LASER ASSISTED NON-SURGICAL THERAPY: LANST

A Thesis

by

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ABSTRACT

Severe, chronic periodontitis is typically treated with mechanical debridement in an effort to gain clinical attachment and hopefully alter etiological factors. If left undisturbed, the plaque biofilm will progressively transform to have a detrimental effect on the periodontium. The search for an effective adjunct to aid in mechanical debridement has lead to the use of lasers. This is supported by recent marketing with a focus in the dental market as well as numerous, recent reports on their range of uses in the dental literature. This paper presents a novel approach to the treatment of severe, chronic periodontitis utilizing the carbon dioxide (CO_2) laser in combination with scaling and root planing for non-surgical therapy. This laser study presents the clinical and bacterial findings of 14 patients compared in a split-mouth design and followed for 6 months. Within the confines of this six month study, sites treated with the laser assisted non-surgical therapy (LANST) tended to show a greater decrease in probing depths and greater gains in clinical attachment levels; however, the results were not statistically significantly better than scaling and root planing alone. The decrease in several suspected periodontal pathogens for the first 3 and 6 months after therapy appears very promising. To the authors' knowledge, this is the first reported case series utilizing a unique ablative CO_2 laser handpiece for sulcular decontamination in combination with scaling and root planing for the treatment of chronic periodontitis.

DEDICATION

This thesis is dedicated to my grandparents and parents for their encouragement and support. To my wife, Kelsey, and daughter, Hadleigh Jean, thank you for your saintly patience and love throughout this entire process.

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NOMENCLATURE

AAP	American Academy of Periodontology
AA	*Aggregatibacter actinomycetemcomitans*
BOP	Bleeding on Probing
CAL	Clinical Attachment Level
CR	*Campylobacter rectus*
CS	*Capnocytophaga* species (*gingivalis, ochracea, sputigena*)
CEJ	Cemento-enamel junction
CO_2	Carbon Dioxide
EC	*Eikenella corrodens*
Er,Cr:YSGG	Erbium, chromium: yttrium, scandium, gallium, garnet laser
Er:YAG	Erbium-doped yttrium aluminum garnet laser
EN	*Eubacterium nodatum*
FN	*Fusobacterium nucleatum/ periodonticum*
LANST	Laser Assisted Non-surgical Therapy
Laser	Light Amplification by Stimulated Emission of Radiation
LPS	Lipopolysaccharides
mm	Millimeters
N/D	Not Detectable
Nd:YAG	Neodymium-doped yttrium aluminium garnet laser
ng	Nanogram

nm	Nanometer
PM	Peptostreptococcus micros
PG	*Porphyromonas gingivalis*
PI	Prevotella intermedia
PD	Probing Depth
S/RP	Scaling and Root Planing
TF	*Tannerella forsythia*
TD	*Treponema denticola*
Vs.	Versus

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I. INTRODUCTION AND LITERATURE REVIEW

Since mankind's inception, the body has been plagued by numerous diseases that incorporate any alteration from the healthy state. The gateway into the body or oral cavity is no exception. There are many indications that any disease of the supporting structures of the dentition or periodontal disease was observed throughout man's known accounts of history [1]. As in many facets of life, when one question is answered concerning a disease, another two are created. This review will focus on a brief review of the histology of the periodontium, the etiology of periodontitis, and treatment thereof. The prime directive for the review of periodontal treatment will be limited to non-surgical therapy, focused mainly at debridement by both manual and mechanical means. In conjunction with a brief overview of guided tissue regeneration principles, the use of lasers to treat periodontal disease will be illustrated as well.

I.2 Histology of the Periodontium

I.2.1 Bone

The main supporting structure for the dentition is the jawbone itself. Although the portions of the bone can be separated in an anatomic sense for classification, the entire process functions as a unit to support the dentition. All bone surfaces are covered by layers of differentiated osteogenic connective tissue. The 10-12 cells thick periosteum, covering the outer surface of bone, consists of the reticular (outer) and cambium (inner) layers. The reticular layer is rich in neurovasculature and largely composed of collagen fibers and fibroblasts.

The periosteum is bound by bundles of periosteal collagen fibers that penetrate into the bone. Cambium is composed of osteoblasts surrounded by osteoprogenitor cells [2]. Tissue lining the internal bone cavities is called endosteum and is composed of a single layer of osteoblasts with occasional small amounts of connective tissue.

The mandible and maxilla are subdivided into a basal bone which is the apical portion which is unrelated to the teeth and the alveolar process. The alveolar process is subdivided even further into three main components: cortical bone, alveolar bone proper, and cancellous trabeculae. The cortical bone is located on the external surface of the jaw and is formed by haversian bone and compact bone lamellae. The blood supply to this occasionally thick bone comes from the periosteum or through Volkmann's canals incorporated in the haverisan system. The alveolar bone proper is analogous to the socket wall and is interpreted radiographically as the lamina dura[1]. This "bundle" bone is characterized by thin, compact bone with a cribriform plate disposition that allows neurovascular bundles and embedded Sharpey's fibers to connect the periodontal ligament to the cancellous bone. Predominantly in the interradicular and interdental spaces, cancellous bone or trabeculae contains a wide variety of irregularly shaped marrow spaces lined with a layer of thin, flattened endosteal cells. Other cellular components that are integral to the formation of bone are the osteoblasts which produce the organic matrix of the bone. The nonmineralized bone matrix or osteoid becomes mineralized through the deposition of calcium, phosphate, sodium, magnesium. These form hydroxyapatite crystals that make up nearly sixty six percent of the composition of bone. The remaining organic portion is mainly Type I collagen with a mix of proteins

such as osteocalcin, osteonectin, bone morphogentic protein (BMP), phosphoprotiens, and proteoglycans[3].

Due to the continual mechanical stresses from mastication and the body's need for calcium ions, bone is constantly remodeling. This requires the large, multinucleated osteoclasts located in Howship's lacunae to release hydrolytic enzymes to digest the organic portions of bone, creating a ruffled border appearance. This constant state of remodeling affects the periodontal ligament which continually adapts to the challenge from everyday function.

I.2.2 Periodontal Ligament

This specialized connective tissue connects cementum to the bone as a type of suspension bridge of intricately interwoven fibers [4]. The terminal ends of these fibers that insert into the cementum are known as Sharpey's fibers or enter into the bundle bone of the socket wall. In squirrel monkeys, studies have shown that during tooth eruption, cemental Sharpey's fibers are the first to appear, followed by Sharpey's fibers emerging from bone [1]. These fibers are also divided into six groups categorized by their location and/or orientation. These groups are transseptal, alveolar crest, horizontal, oblique, apical, and interradicular[5].

Additionally, four types of cells have been identified in the periodontal ligament: connective tissue cells (fibroblasts, cementoblasts, osteoblasts), epithelial rest cells, immune system cells (neutrophils, lymphocytes, macrophages, mast cells, eosinophils), and cells associated with neurovascular elements.[6]

Based on rodent studies, the attachment apparatus is constantly undergoing remodeling. The fibroblast and endothelial cells are active in the deposition and resorption of alveolar bone. The fibroblasts produce new collagen fibers as residual mesenchmal cells differentiate into osteoblasts and cementoblasts (7).

I.2.3 Cementum

The root surface of a tooth is typically covered by a calcified avascular tissue called cementum which was subdivided by Schroeder depending on its characteristics.

"Acellular afibrillar cementum contains neither cells nor extrinsic or intrinsic collagen fibers, apart from a mineralized ground substance. It is a product of cementoblasts and is found as coronal cementum in humans, with a thickness of 1 to 15 μm.

Acellular extrinsic fiber cementum is composed almost entirely of densely packed bundles of Sharpey's fibers and lacks cells. It is a product of fibroblasts and cementoblasts and is found in the cervical third of roots in humans but may extend further apically. Its thickness is between 30 and 230 μm.

Cellular mixed stratified cementum is composed of extrinsic (Sharpey's) and intrinsic fibers and may contain cells. It is a co-product of fibroblasts and cementoblasts, and in humans it appears primarily in the apical third of the roots and apices and in furcations areas. Its thickness ranges from 100 to 1000 μm.

Cellular intrinsic fiber cementum contains cells but no extrinsic collagen fibers. It is formed by cementoblasts, and in humans it fills resorption lacunae.

Intermediate cementum is an ill-defined zone near the cementodentinal junction of certain teeth that appears to contain cellular remnants of Hertwig's sheath embedded in calcified ground substance [1, 7-9]. The less calcified cellular cementum that occurs once the tooth has reached occlusal contact will have lifelong deposition of rapid forming cementum and is typically in the most apical areas of a root [10].

The inorganic content of cementum (hydroxyapatite) is 45% to 50%, which is less than that of bone (65%), enamel (97%), or dentin (70%) [11]. If damage was to occur to the cementum, repairs can be done, but only in the presence of viable connective tissue. If epithelium proliferates into an area of resorption, neither regeneration nor repair will take place.[12] In a form of abnormal repair, teeth with cemental resorption have a fusion of the cementum and alveolar bone with obliteration of the periodontal ligament which can lead to ankylosis. In periodontal disease, the cementum can become a reservoir for inflammation by allowing calculus to attach to it by cuticular attachment, mechanical locking into undercuts, and direct attachment of calculus matrix [13]. The most frequently encountered method of attachment is found to be the apparent melding of calculus matrix to the surface of cementum [13].

I.2.4 Connective Tissue

The connective tissue situated between the periosteum and epithelial lining is a conglomeration of ground substance surrounding fibers made by and sustained by specialized cells. The ground substance is mainly composed of a high volume of water with proteoglycans and glycoproteins. This filling surrounds the fibroblast which is responsible for forming collagen fibrils. The majority of the connective tissue is mainly

Type I collagen which serve as support scaffold when the teeth are in function [1]. Near the basement membrane, or external basal lamina, Type IV and VII collagen, laminin, heparan sulfate proteoglycan, fibronectin, nidogen (entactin), and the proteoglycan perlecan serve as anchorage [14]. Other anchoring fibrils were noted near the coronal portion that mechanically locked into the epithelium. Susi *et al* further suggested that the attachment of epithelium to connective tissue was due to the interlocking arrangement of anchoring fibrils and collagen fibrils from the basement membrane to the basal epithelial cell [15]. Gargiulo *et al* reported that the average mean width of connective tissue attachment was 1.07mm [16].

I.2.5 Oral Epithelium

The purpose of epithelium is to protect the deeper structures while allowing a selective interchange with the environment which is achieved by the proliferation and differentiation of the keratinocyte [1]. Gargiulo reported that the average mean width of the epithelial attachment was 0.97mm [16]. The oral epithelium is composed of four distinct layers. The deepest layer or the stratum basale is the layer for the synthesis of new cells. These cuboidal cells lay along the basement membrane and are attached to each other via gap junctions and hemidesmosomes. These hemidesmosomes also fasten the epithelial cells to the basement membrane. The hemidesmosomes play a critical role since they also form the attachment of a specialized basement membrane to a tooth surface.[17] The second layer or stratum spinosum derives its name from the microscopic appearance of peripheral cytoplasmic processes which resemble tiny spines. This layer also contains modified lysosomes known as keratinosomes or Odland bodies, which

contain a large amount of acid phosphatase, an enzyme involved in the destruction of organelle membranes, which occurs suddenly between the next two layers, the granulosum and corneum strata [1]. The third layer is critical to the process of keratinization. Known as the granular cell layer, it is characterized by the presence of keratohyalin granules and gradual flattening of the cell structure. Finally, the stratum corneum is a keratinized cell layer that makesup the outermost layer of the epithelium [1].

The epithelium also houses many other specialized cells. For example, melanocytes secrete melanin for pigmentation of the epithelium and the dendritic Langerhans cells aid the immune system as sentry posts [18]. Merkel cells act as touch-sensory cells [19]. All these cells are continuously being replicated. The mitotic activity exhibits 24-hour periodicity, with highest and lowest rates occurring in the morning and evening.[20] Typically, the turnover for the gingiva is between 10 and 12 days, a key point that will be discussed later.[21,22].

I.2.5.1 Junctional Epithelium

This highly specialized stratified squamous nonkeratinizing epithelium creates a collar around the teeth located from the cemento-enamel junction (CEJ) to the free gingival margin. It is initially developed by the union of the oral epithelium and the reduced enamel epithelium during tooth eruption. However, the reduced enamel epithelium is not essential because the junctional epithelium will develop *de novo* after pocket curettage, pocket elimination surgery, and around an implant. [23-25]

At the coronal portion it is typically 15-30 cells thick and tapers apically to 1 to 3 cells where it will then connect to the tooth surface, creating a biological seal from the

oral cavity [14]. Ideally, the seal will extend from the CEJ to the gingival margin, approximately 2 millimeters (mm) in height [16]. However, the oral cavity will always have sub-clinical signs of inflammation so the typical length ranges from 0.25 to 1.35 mm [1, 26]. Therefore, the coronal termination of the junctional epithelium typically corresponds to the apex of the gingival sulcus. The junctional epithelium typically exhibits a renewal rate of 5-6 days.[21,22]

The junctional epithelium is composed of only two layers: the basal (stratum basale) and suprabasal (stratum suprabasale) [14]. The cuboidal shaped basal cells line the gingival connective tissue. The flat suprabasal cell layer is oriented parallel to the tooth surface and are also called DAT cells (directly attached to the tooth) [27]. These cells form and maintain the 'internal basal lamina' that faces the tooth surface. While the internal basal lamina or external basement membrane resembles other basement membranes found between epithelium and a connective tissue, the internal basal lamina lacks most of the common basement membrane components such as collagen types IV and VII [28]. The internal basal lamina together with hemidesmosomes forms the interface between the tooth surface and the junctional epithelium [29, 30]. The lamina densa directly faces the enamel, dentin, or cementum [14]. This attachment mechanism has also been demonstrated to exist on a dental calculus layer in a bacteria-free environment [31].

The intercellular spaces of the junctional epithelium provide a pathway for fluid and transmigrating leukocytes. In a healthy human oral cavity, approximately 30,000 polymorphonuclear leukocytes migrate per minute through the junctional epithelia [32]. The tissue fluid secreted through the junctional epithelium represents a defense system against

bacterial challenge. This is interpreted as gingival fluid that is an exudate and its flow rate corresponds to the degree of inflammation.[14]

The collaboration of many studies illustrate the form and function of the non-keratinizing junctional epithelium as a fast-renewing, non-differentiating cell type that forms a biological seal that can easily serve as a pathway for inflammatory cells to become direct contact with any bacterial invasion from the oral cavity [25].

I.2.5.2 Sulcular Epithelium

This thin, nonkeratinized, stratified squamous epithelium is situated as the connecting layer from the coronal aspect of the junctional epithelium to the crest of the gingival margin. If exposed to the oral cavity or through elimination of the bacterial flora, the sulcular epithelium has the potential to keratinize.[22, 33] Conversely, the outer epithelium loses its keratinization when it is placed in contact with the tooth.[22] This feature serves as a semipermeable membrane allowing the body's own natural defenses to attack bacterial products that pass into the gingiva [34]. Gargiulo reported that the average mean width of sulcus depth was 0.69mm.[16]

I.2.5.3 Marginal or Free Gingiva

The marginal, or unattached, gingiva is typically about 1 mm wide, forming a portion of the gingival sulcus. This "collar" represents the terminal edge or border of the gingiva and in can be distinguished from the adjacent, attached gingiva by a shallow, linear depression known as the free gingival groove.[1]

I.2.5.4 Attached Gingiva

Orban described the attached gingiva as tightly bound to the underlying bone that is typically firm, dense, and stippled similar to an orange peel. However, in regards to width and thickness, Bowers and Goaslind both noted extreme ranges dependent on subjects and regions of the mouth. Some generalized statements that could be extrapolated from Bowers were that the maxilla usually exhibited a broader zone of attached gingiva than the mandible. Also, the width of attached gingiva was greatest in the incisor region (especially the lateral incisor) and the least in the canine and first premolar sites[35]. Derived from Goaslind's study: the free gingiva averaged 1.56mm in thickness, the attached gingiva averaged 1.25mm in thickness, and an overall mean thickness for all areas was 1.41mm.[36].

I.3 Gingivitis & Periodontitis

The American Academy of Periodontology defines plaque induced gingivitis as inflammation of the gingiva, but without clinical attachment loss [37]. Periodontitis is the progressively destructive inflammation of the supporting tissues of the teeth leading to loss of bone and periodontal ligament [37]. The primary etiology of periodontitis is the accumulation of plaque or bacterial biofilm and the host's immune response to said plaque.

In 1977, Page and Schroder categorized the stages of gingivitis based on histological characteristics. After plaque accumulation that has been undisturbed for 2-4 days, the "initial lesion" has mild vasculitis, loss of perivascular collagen, and the junctional epithelium experiences an increased migration of leukocytes, alteration of its

coronal portion, and a clinical increase in crevicular fluid output. This stage is considered subclinical gingivitis. 4-7 days after plaque accumulation, the lesion progresses to the "early lesion" stage where there is evidence of vascular proliferation with rete peg formation which appears clinically as erythematous gingival margins. This is followed by lymphocyte infiltration with additional collagen loss. At the 2-3 week mark, the lesion is referred to as the "established lesion" which may remain stable for extended periods of time. The key features of the established lesion are the presence of plasma cells and bleeding on probing. All tissue damage up to this point is still reversible and considered gingivitis only. As the lesion progresses to the next stage, the "advanced lesion," there is periodontal pocket formation, surface ulceration and suppuration, and destruction of the alveolar bone and periodontal ligament. Although irreversible, the progression of the disease can be stopped and the health of the gingiva stabilized [38].

The first indications of irreversible, destructive disease are the development of a pocket between the tooth surface and gingiva with perceptible apical displacement of the junctional epithelium forming a "long" junctional epithelium. Epithelium along the cementum/soft tissue interface prevents the establishment of connective tissue reattachment and results in an impaired attachment apparatus [39].

I.4 Bacterial Influence on the Periodontium

Periodontal health can be considered to be a state of balance when the bacterial population coexists with the host and no irreparable damage occurs to either. Disruption of this balance causes alterations in both the host and biofilm bacteria and results in the destruction of the connective tissues of the periodontium [1]. Dental plaque is a biofilm

initially formed through bacterial interactions with the tooth and among different species. Furthermore, the bacteria found in the plaque biofilm are influenced by external environmental factors including absence of oral hygiene and the host's immune system.

Plaque formation is initiated by glycoproteins from saliva that initially coats a clean tooth surface and becomes incorporated into the developing plaque biofilm. Other organic constituents of the matrix include polysaccharides, proteins, and lipid material. Plaque is composed primarily of microorganisms. One gram of plaque (wet weight) contains approximately 2×10^{11} bacteria [40]. Calcium and phosphorus with trace amounts of sodium, potassium, and fluoride constitute the inorganic component of plaque. As the inorganic content of plaque, primarily of calcium phosphate mineral salts, increases, the plaque can harden to become calculus [41].

The formation of dental plaque has an ordered and predictable ecologic succession in that there is a transition from gram-positive, early colonizers to gram-negative secondary colonizers. The process of plaque formation can be divided into three phases: formation of the pellicle coating on the tooth surface, initial colonization by bacteria, and secondary colonization and plaque maturation [1].

Derived from saliva, crevicular fluid, bacterial and host tissue cell products and debris, the composition of the pellicle varies dependent on the surface. Pellicles function as a protective barrier, providing lubrication for the surfaces and preventing tissue desiccation. However, they also provide a substrate to which bacteria in the environment attach [42].

The difficulty of periodontal disease is that the periodontal microflora is extremely diverse [43, 44]. From Socransky's work utilizing whole genomic DNA probes and checkerboard DNA-DNA hybridization to assess 13,261 plaque samples in 185 patients, a series of complexes were found to correlate well with the type of bacteria that colonize the biofilm [45]. The early colonizers are either independent of the defined complexes or members of the yellow (*Streptococcus* species) or purple complexes (*Actinomyces* species). These aerobic early colonizers lower the reduction-oxidation potential of the environment, facilitating the growth of anaerobic species [46, 47].

Green, orange or red complexes have a propensity to be secondary colonizers. The green complex includes *Eikinella corrodens, Aggregatibacter actinomycetemcomitans* serotype a, and *Capnocytophaga* species. The orange complex includes *Fusobacterium, Prevotella*, and *Campylobacter* species. The red complex (*Porphyromonas gingivalis, Tannerella forsythia, and Treponema denticola*) is associated with bleeding on probing, an important clinical parameter of destructive periodontal diseases [1, 45].

In 1976, Loesche defined two hypotheses about plaque. According to the nonspecific plaque hypotheses, the toxins released by plaque's biomass can be neutralized by the host. However, the host's defenses can be overwhelmed as the amount of plaque increases. The theory states that control of the plaque accumulation will control periodontal disease.

The specific plaque hypothesis states that only a small subdivision of plaque is pathogenic, and its pathogenicity depends on the presence of or increases in specific

microorganisms [48]. This concept predicts that plaque harboring specific bacterial pathogens results in periodontal disease because these organisms produce substances that mediate the destruction of host tissues [1]. Support for this theory includes data showing that cultivation of plaque microorganisms from sites of chronic periodontitis reveals high percentages of anaerobic (90%) gram-negative (75%) bacterial species [49, 50]. Additional quantification have consistently revealed elevated proportions of spirochetes [51, 52]. In chronic periodontitis, the bacteria most often cultivated at high levels include *P. gingivalis, T. forsythia, P. intermedia, C. rectus, Eikenella corrodens, F. nucleatum, A. actinomycetemcomitans, P. micros*, *Treponema,* and *Eubacterium* species. [53-59].

Dzink *et al* found from plaque samples of 33 patients that when periodontally active sites (i.e., with recent attachment loss) were examined in comparison with inactive sites (i.e., with no recent attachment loss*), C. rectus, P. gingivalis, P. intermedia, E. nucleatum,* and *T. forsythia* were found to be elevated in the active sites [60]. Disease progression was associated with detectable levels of *P. gingivalis, P. intermedia, T. forsythia, C. rectus,* and *A. actinomycetemcomitans* [60, 61] and elimination of the same specific bacterial pathogens with therapy is associated with an improved clinical response [62-64].

I.5 Goals of Periodontal Therapy

Although the gold standard for successful treatment of chronic periodontitis is a gain in clinical attachment level, other clinical goals such as complete debridement of the root surface, regeneration of periodontal structures, and patient preference for esthetics must also be considered as credible end points [65].

There are three modes of healing after periodontal therapy. The first being *new attachment* which is defined as the adherence of connective tissue or epithelium with a root surface that has been deprived of its original attachment apparatus. This new attachment may be epithelial adhesion and/or connective tissue adaptation or attachment and may include new cementum [37]. This is not to be confused with *reattachment* which is to attach again or the reunion of epithelial and connective tissue with a root surface[37]. The defining factor between the two is the root surface being considered "diseased" or not. For example, consider a patient treated with periodontal therapy via open flap debridement. The root surfaces that were previously calculus laden are now clean and the tissue is adapted over it in hopes to gain new attachment. If the same person had a few healthy teeth, but in order for the surgeon to gain better access, the gingiva of those teeth were included into the flap design. These teeth did not have clinically diagnosed disease and thus the re-approximation of the flap would be the reunion of the epithelial and connective tissue with the root surface. The same patient may have a few areas where periodontal disease lead to destruction of the alveolar housing and therapy indicates to allow the site to heal by *repair*. This is the characteristic healing pattern for resective type of surgeries where the healing of a wound does not fully restore the architecture or function[37].

The primary goal of any periodontal procedure is the formation of a new clinical attachment (cementum, periodontal ligament, bone, and connective tissue). The ultimate, yet typically elusive, goal of any periodontal therapy is aimed at *regeneration* or the reproduction or reconstitution of a lost or injured part. This topic is further divided into

guided tissue regeneration or *periodontal regeneration.* Guided tissue regeneration procedures are attempting to regenerate lost periodontal structures through differential tissue responses. Guided bone regeneration typically refers to ridge augmentation or bone regenerative procedures; guided tissue regeneration typically refers to regeneration of periodontal attachment. Barrier techniques, using materials such as expanded polytetrafluoroethylene, polyglactin, polylactic acid, calcium sulfate and collagen, are employed in the hope of excluding epithelium and the gingival corium from the root or existing bone surface in the belief that they interfere with regeneration. Periodontal regeneration is the restoration of lost "periodontium" without the use of a barrier membrane [37].

During the healing stages of a periodontal pocket, the area is invaded by cells from the oral epithelium, gingival connective tissue, bone, and periodontal ligament. The final outcome of periodontal pocket healing depends on the sequence of events during the healing stages [66]. If the epithelium proliferates along the tooth surface, the result will be a long junctional epithelium. If the cells from the gingival connective tissue proliferate into the area first, the result is fibers parallel to the tooth surface and with remodeling of the alveolar bone without attachment to the cementum. If bone arrives first, root resorption and ankylosis may occur. Cementum and the periodontal ligament will only form when cells from the existing periodontal ligament proliferates coronally along the root surface [66].

I.6 Non-surgical Periodontal Therapy

The aim of non-surgical therapy is to eliminate the microbial biofilm and calcified deposits from diseased root surfaces through root surface debridement [67]. A roughen root surface facilitates attachment and accumulation of bacterial biofilm [68-70]. The rough calculus surface may not, in itself, induce inflammation, but may serve as an ideal substrate for subgingival microbial colonization [67]. This is also supported by the fact that attachment has been demonstrated to exist on a dental calculus layer in a bacteria-free environment [31]. However, due to its porous nature, calculus serves as a reservoir for bacterial products such as endotoxin that elicit an inflammatory response with subsequent tissue damage. First coined by Pfeiffer, endotoxin is a toxin integrated into a bacterial cell that is released after destruction of the cell wall [71]. Later, this endotoxin would be defined as lipopolysaccharide (LPS), a major component of the Gram negative bacterial outer membrane. This was noted in the periodontal literature when in periodontitis, an inhibitory substance was found to penetrate the surface of cementum which prevented the growth of epithelial cells in tissue culture.[72] The following year, Schwartz *et al* suggested that this inhibitory substance, "endotoxin," could also penetrate healthy, intact crevicular epithelium [73]. Caffesse and Nasjleti suggested that bacterial collagenase penetrates through intact gingival epithelium with bacterial hyaluronidase being the potentiating factor [74]. That same year, Aleo reported the presence of endotoxin in the cementum that in a dose dependent matter, lead to the inhibition of the proliferation of cultured mouse fibroblasts.[75] However, a decade later, from Nyman's 1986 histological study on canines, the toxins are found concentrated on the root surface. If any endotoxin

has penetrated into the surface, it was not detrimental to accomplishing periodontal health as the canines recovered with a junctional epithelium without inflamed connective tissue [76]. This was further supported by a human clinical study by Nyman *et al* in 1988. Utilizing a split mouth design in eleven patients, the authors compared scaling and root planing of teeth where all the cementum was removed versus without cementum removal. After a period of 2 years, both sides showed the same degree of improvement, including gain of clinical attachment [77]. Therefore, treatment must be aimed at remove of the endotoxin without extensive removal of the underlying cementum [76, 78].

Nonsurgical therapy has incorporated several adjunctive treatment options which include: debridement by manual or mechanical means, supragingival and/or subgingival irrigation, local drug delivery, systemic antibiotics, host-response modulation, photodynamic therapy, and lasers. As the dental community has noted, no method is one hundred percent effective, even when various combinations and adjuncts have been deployed. The majority of the results are influenced by the complexity of the biofilm. For example, the resistance to antimicrobials may relate to limited diffusion of substances into the biofilm matrix, the slow rate of cell growth in the biofilm environment, and possibly to altered properties of bacteria in response to growth on a surface [79].

I.6.1 Effects of Oral Hygiene

Considering that elimination of bacterial deposits can resolve inflammation and arrest disease progression, does an improvement of oral hygiene improve the periodontal status alone? With professional daily supragingival prophylaxis for three weeks, a significant reduction of facultative and obligatory anaerobes was noted [80]. When twelve

patients were subjected to professional supragingival plaque control 3 times per week for 3 weeks, Hellstrom *et al* noted significant improvements in furcations, suprabony defects, and clinical gingival health, but notes that there were no significant improvements in infrabony defects. Out of the twenty sites over five millimeters in depth, only five improved but never surpassed four millimeters in depth [81]. Kho *et al* found similar results and stated that oral hygiene may have no impact when the probing depths exceeded five millimeters [82]. This is supported by Beltrami *et al* that subjected eight moderate to severe periodontitis patients to a professional supragingival prophylaxis three times a week for three weeks [83]. They noted no improvement in probing depths over 6.5 mm. In a study of forty seven patients with interdental bleeding, Caton *et al* wanted to determine the effect of a wooden interdental cleaner (Stim-U-Dent) compared to subgingival scaling. The patients were divided into three groups. Group I bled upon stimulation with the interdental cleaner. Groups II and III bled initially but were converted to non-bleeders with oral hygiene alone (Group II) or with oral hygiene combined with subgingival scaling (Group III). Histology indicated that Group III had the most improvement in regards to reduction of inflammation of the connective tissue. However, the coronal location had significantly less inflamed connective tissue than the apical location in all three groups [84]. The authors concluded that although oral hygiene reduced interdental inflammation, subgingival scaling in addition to oral hygiene decreased the interdental inflammation to a greater extent within four weeks. Cercek *et al* followed 7 patients diagnosed with generalized chronic periodontitis. The patients underwent phases of treatment where the phase continued until no improvement in

bleeding scores of probing depths was noted over 3 successive examinations. Phase 1 consisted of tooth brushing and flossing, Phase 2: subgingival use of the Perio-Aid (an oral anti-septic), and Phase 3: supra and subgingival instrumentation. Patients had minimal improvement after Phase 1, but what improvements that were made, were maintained by Phase 2. After Phase 3, the authors noted pronounced improvement in all clinical parameters. The authors concluded that home care procedures alone could not expect significant improvement in periodontal pockets. The results of their study concluded that instrumentation accounted for the majority of improvement seen after a combined therapy of both plaque control and instrumentation [85].

Although there is a reduction in the clinical parameters commonly associated with gingivitis, a minimal effect is noted on the clinical parameters associated with periodontitis. Bacterial composition in probing depths over five millimeters cannot predictably be altered, so subgingival mechanical instrumentation is essential in conjunction with adequate personal oral hygiene [86].

I.6.2 Effectiveness of Scaling and Root Planing with Surgical Access

I.6.2.1 Non-Molar Sites

In a clinical investigation of thirty three patients who presented with 3 adjacent buccal non-molar surfaces having similar pocket depths (mean = 3.89 mm). One tooth was treated by hand instrumentation prior to flap reflection, one treated after flap reflection and one served as a control. After debridement and Gentian violet staining, the roots were photographed. The roots were then re-instrumented until they were judged to be clean by the operator. The roots were stained and photographed again. Although less

staining was evident on surfaces treated by open flap root planing vs. closed flap root planing, no surface was completely devoid of staining. The authors concluded that open debridement was more effective than closed debridement for removing stainable material from buccal root surfaces [87]. This must be taken with caution as an instrumented root surface with residual fibrin and debris will adsorb stain, which can be misinterpreted as bacterial accretions [88].

I.6.2.2 Molar Sites

Caffesse *et al* evaluated the effectiveness of SC/RP with and without flap access on calculus removal in twenty one patients that were slated for immediate dentures. Six teeth were assigned one of the following treatments: two teeth received scaling and root planing without flap access, two received scaling and root planing with flap access, and two received no treatment to serve as controls. The teeth were extracted and residual calculus was quantified under a stereomicroscope with a micrometer. The authors noted that calculus retention was most common around the CEJ along with grooves, fossae, furcations, and areas apical to restorations. No difference was noted in posterior versus anterior teeth when comparing residual calculus, but the probability of leaving calculus increased as probing depth increased. When comparing SC/RP alone vs. SC/RP with a flap, the percentage of completely calculus free surfaces was 86% for both treatments at 1-3 mm sites, 43% vs. 76% at 4-6 mm sites, and 32% vs. 50% at greater than 6 mm sites, respectively. The authors concluded that SC/RP alone and SC/RP with a flap are equally effective for calculus removal in pockets less than 3 mm. However, SC/RP with a flap is significantly more effective for calculus removal in pockets greater than 3 mm [89].

In 36 patients with 61 molars with a hopeless prognosis and probing depths greater than 6 mm, Fleischer *et al* compared if the effectiveness of SC/RP of multi-rooted teeth is superior by surgical access and/or operators whom were either periodontal residents or periodontists. After SC/RP with or without a flap, the molars were extracted and sectioned so that the furcation dome could be examined. If the probing depth was less than 4 mm, no difference was found. For the sites greater than 4 mm, the periodontist was more effective in all aspects, especially with an open approach. However, even with this approach, only 68% of the furcation surfaces treated by the periodontists were calculus free. Residual calculus was found most often at furcation entrances, external and furcation line angles, just below the CEJ and in root concavities. The authors' concluded that surgical access and operator experience significantly enhanced calculus removal in molars with furcation invasion. However, complete calculus removal from molar furcations is not predictable when conventional hand and ultrasonic instruments are utilized [90].

Wylam *et al* compared the effectiveness of SC/RP on multirooted teeth using a closed versus an open flap approach in 60 molars which were assigned to one of three groups: untreated controls, closed approach, and open flap approach. Afterwards, the teeth were extracted, stained with methylene blue, and examined for stained residual deposits. The authors noted the untreated teeth had 91% of root surfaces covered in deposits, closed approach had 54.3%, and the open approach had 33%. Comparisons showed no difference between shallow or deep probing depths or between techniques with regard to staining of furcation deposits. There was no correlation between the time

spent in root debridement and the percent residual deposit in the area. The authors concluded that hand instrumentation alone is inadequate for thorough debridement of the furcations of multi-rooted teeth and suggest the use of adjuncts to aid in root debridement [91]. Again, this must be considered with caution as an instrumented root surface with residual fibrin and debris will adsorb stain, which can be misinterpreted as bacterial accretions [88].

I.6.2.3 Furcation Sites

In 1986, Matia and colleagues compared the effectiveness of surgical vs. nonsurgical accessed scaling and root planing as well as hand vs. ultrasonic instruments for the removal of calculus from 50 mandibular molar degree II or III furcations. The teeth were divided into the following groups with 10 teeth per group: surgical S/RP with hand curettes, nonsurgical S/RP with hand curettes, similar but with ultrasonic instruments, and a non-treated control. The teeth were then extracted and quantification of residual furcal calculus was made under a stereomicroscope with an ocular grid. Concerning the furcal dome, both nonsurgical methods had comparatively lower percentages of calculus-free surfaces. With surgical access, both hand and ultrasonic instrumentation was able to clean wide furcations thoroughly. In narrow furcations, ultrasonic instrumentation has an advantage. Another finding was that the operators were unable to differentiate between burnished calculus and cementum. They concluded that the use of an open flap approach and ultrasonic instruments seems indicated for the debridement of narrow furcations [92].

In a two part study, Parashis and colleagues looked at calculus removal in mandibular molars with and without surgical access. Both studies involved 30 "hopeless" molars with II or III degree furcation involvement that were treated either with a closed approach, surgical access, or rotary diamond instrumentation in the furcation areas. The teeth were extracted and analyzed with a stereomicroscope to determine the remaining calculus on external and internal surfaces. Sites with probing depths greater than 7 mm had more residual calculus for all groups. The most effective method to remove calculus in the furcation was a rotary diamond with 5% surface area still calculus laden compared to the 60% left by a closed technique. Surgical access to the furcation improved the calculus free area only by 10%, but reduced residual calculus in the flutes by half (70% closed vs. 35% open). In the second part of the study, the authors divided the furcations into narrow and wide furcations (2.4 mm being the boundary). The authors noted that rotary diamonds were the most effective in both furcations, especially the wide ones. The authors also noted that even with surgical access, complete calculus removal was not obtainable. A rotary diamond must be used with caution in the furcation because of potential increased sensitivity from excessive cementum removal and potential accessory canals [93, 94]. Leon and Vogel compared the effects of hand scaling with ultrasonic debridement in furcations using dark-field microscopy. 33 furcated molars were scaled by either hand instruments or ultrasonic scalers and examined. They found that ultrasonic debridement was more effective in Class II and III furcations at reducing spirochetes and motile rods. Curettes were typically at least 1 mm wide, whereas the roof of furcations are often less than 1 mm [95]. It was

suggested this size discrepancy prohibited effective debridement [95, 96]. Leon and Vogel speculated that ultrasonic scalers negotiated the Class II and III furcations better although no difference was noted between instrumentation modalities in Class I furcations.

I.6.3 Clinical Effectiveness of Scaling and Root Planing Alone

I.6.3.1 Non-Molar Sites

Badersten *et al* completed a series of studies looking at the long term effects of nonsurgical periodontal therapy in moderate to severe periodontitis. In a split mouth design of 15 patients, non-molars were treated by hand or ultrasonic instrumentation. During the first four weeks, the patients returned for oral hygiene appointments which were repeated as needed from 1-7 months. Instrumentation was repeated at 1, 3 and 7 months after baseline after clinical parameters were assessed. A significant reduction of gingivitis associated parameters decreased by 4 weeks. Bleeding on probing decreased 4 weeks after instrumentation and the noted mean total probing depth reduction was 1.3-1.7 mm. A mean recession of 1.4-1.6 mm was noted with the majority of the recession taking place within the first two months. Deep pockets (greater than 4 mm) tended to gain attachment (~1.5mm) and shallow pockets (less than 4 mm) tended to lose 1.5 mm of attachment. Their major conclusion were that with hand or ultrasonic instrumentation, moderate periodontitis (4-7.5 mm probing depths) around non-molar teeth can be maintained with excellent oral hygiene [97]. A second study to address patients with severe periodontitis around non-molar teeth was treated in the same manner. This study concluded that surgical therapy should be postponed for 6-9 months after S/RP to allow the gingiva to heal to its full potential. A study releasing the 4 year results of the

previous studies showed that minimal changes in probing depths, bleeding on probing, and clinical attachment were seen independent of the initial probing depth. Between the second and fourth year, any sites that exhibited attachment loss was typically different than the sites that lost clinical attachment during the first two years. This leads one to the conclusion that, regardless of treatment and oral hygiene, deeper probing depths are harder to maintain [98]. Badersten and colleagues attempted to determine if bleeding on probing, suppuration, or residual probing depth may provide any insight if a site was more likely to breakdown in the future. Unfortunately, not a single combination of factors provided a "positive predictive value" or sensitivity and diagnostic predictability of future loss of attachment around non-molars in patients with adequate oral hygiene [99].

I.6.3.2 Molar Sites

Nordland *et al* examined the effects of plaque control and root debridement in non-molar, molar flat surface, and molar furcation sites in 19 generalized periodontitis subjects with at least 2 molars with furcation involvement. A single of episode of full mouth, closed SC/RP with ultrasonic and hand instruments with an average time of 3.2 minutes per tooth on non-molars and 6.7 minutes per tooth on molars. Scaling, polishing and isolated root debridement of residual deep or bleeding sites was accomplished at 15, 18 and 21 months. All sites initially less than 3.5mm showed minimal PD changes. The majority of furcation sites with initially greater than 7mm had higher bleeding scores throughout the study and exhibited no gain in attachment at any time and showed a mean loss of attachment of 0.5mm at 24 months. Mean probing depth decreased in the 4-6.5mm group by 3 months and maintained throughout the 24 month period. Any gains of

attachment in the 4-6.5 mm non-molar and molar flat surface sites that were observed at 6 months had relapsed by the 24 month exam. Overall, molar furcation sites with an initial probing depths greater than 4.0 mm showed a poorer response to plaque control and debridement compared to non-molar and molar flat surface sites with similar initial probing depths [100].

I.6.3.3 Full Mouth Considerations

In 1980, Morrison *et al* reported on the short term effects of initial, nonsurgical periodontal treatment in 90 patients. After collecting baseline data, the patients received scaling and root planing, oral hygiene instructions, and an occlusal adjustment. After 4 weeks, the patients returned for a re-evaluation. They noted that the greatest improvement of clinical parameters occurring in sites that had initial probing depths greater than 7 mm. These site improved by 2.2 mm in regards to probing depth with a 0.91 mm gain in attachment. The sites that initially measured 4-6 mm saw an improvement by 0.96 mm in probing depth and 0.23 mm gain in attachment. Sites that initially started in the 1-3 mm range saw a minor decrease of 0.17 mm in probing depth, but no change to attachment levels. The author concluded prior to assessing the need for surgical therapy, that the hygienic phase of periodontal therapy should be accomplished since it can result in a decrease of gingival inflammation and probing depths (especially in deeper pockets) [101].

In 1989, Loos *et al* evaluated the clinical effects of root debridement in molars and non-molars with 2 year follow up. After baseline measurements of plaque index, BOP, probing depth, and attachment levels were taken, the 12 patients received full

mouth root debridement with an ultrasonic scaler. The patients returned every 3 months for measurements, oral hygiene reinforcement, and supragingival prophylaxis. Periodontal sites were grouped into molar furcation sites, molar flat-surface sites and non-molar sites. Mean plaque scores remained around 20-40% and bleeding scores decreased in shallow sites only. In non-molar teeth, the majority of the moderately deep and deep sites showed probing depth reduction and gain in attachment level. However, moderately deep and deep sites in molar furcations showed limited initial probing depth reduction and tended to rebound to baseline depths. In probing depths greater than 7 mm, an initial gain of probing attachment was seen for all categories of sites. While non-molar sites retained this gain, the corresponding molar furcation site regressed. Overall 25% of molar furcation sites demonstrated probing attachment loss as compared to 7% for non-molar sites and 10% for molar flat-surface sites. The authors' conclusion was that inaccessibility to the furcation as well as concavities and other root surface irregularities limit the efficacy of root debridement [102].

Lindhe *et al* performed a split mouth design to evaluate surgical vs. non-surgical therapy in 15 patients with advanced periodontitis. After a baseline exam, 2 quadrants received a modified Widman flap with S/RP while the other two quadrants received only S/RP. Patients came back every two weeks for prophylaxis for 6 months after the final operation. Afterwards, the patients had a prophylaxis every 3 months until the patient completed 2 years post operative. In this patient pool, both treatments were comparable. The authors concluded that both therapies prevented further attachment loss, even gain in deeper probing depths [103].

I.6.4 Clinical and Microbiological Responses

I.6.4.1 Clinical Improvements

Tagge *et al* in 1975 did a study in regards to using adjacent teeth for histological analysis before and after treatment. In 22 patients, three clinically similar buccal or lingual sites on adjacent teeth were selected. Using bleeding, edema, and crevicular fluid (presence or absence), each tooth was assigned a gingivitis score. These scores correlated as: 1 was normal, healthy gingiva, 2-4 was mild, 5-7 was moderate, and 8-10 was considered severe gingivitis. For each pocket, one was designated as a control which was biopsied immediately for baseline, one received only oral hygiene, and the final was planed to a smooth, hard surface to the depth of the pocket. The hygiene and S/RP were both biopsied about 60 days later. Correlating histological findings with clinical parameters, non-treated teeth had gingiva with edema that bled easily on probing and over 50% of the gingival fibers were replaced with inflammatory infiltrate. Teeth subjected only to oral hygiene had a continuous band of chronic inflammatory cells between epithelial rete ridges that had delayed bleeding on probing. The S/RP group had sparse chronic inflammatory cells that were confined immediately adjacent to sulcular epithelium and had none to minimal bleeding to probing [104].

I.6.4.2 Bacterial Alterations

I.6.4.2.1 Hand Instrumentation

Shiloah and Patters evaluated the effects of scaling and root planing on periodontal pathogens in 7 patients with moderate to severe periodontitis. Bacterial samples to specifically look for *Actinobacillus actinomycetemcomitans*, *Porphyromonas*

gingivalis, and *Prevotella intermedia* were collected prior to, 1 week after, and 4 weeks after S/RP with curettes and an ultrasonic. Following treatment, each quadrant was assigned to receive either intra-pocket irrigation with saline, tetracycline, chlorhexidine, or nothing (control). All clinical parameters were noted to improve significantly, but no differences were found between treatments rendered. Therapy resulted in reduction of the overall number of species below detectable levels in 75% of the sites by 1 month with *Prevotella intermedia* being the most prevalent organism found in the group of patients. The authors concluded that while S/RP is effecting in reducing the bacterial load, a single episode of subgingival irrigation did not have a major effect [105].

In a follow up study, Shiloah and Patters studied the repopulation of the periodontal pathogens in the absence of supportive therapy for one year. In the same group of patients, focusing in on probing depths greater than 5 mm, clinical and microbial analyses were recorded at 1 week, and 1,3, 6, 9, and 12 months post therapy. They noted no difference between the groups, but did report that half or less of the sites became re-infected at 12 months. They concluded that a single episode of pocket irrigation with antimicrobial agents following S/RP did not affect the rate of repopulation of periodontal pockets. S/RP does have a suppressive effect on the observed pathogens for a majority of the sites, but the presence of Aa, Pg, and Pi may be a risk factor for reoccurrence without periodontal maintenance therapy [106].

Magnusson *et al* studied the subgingival microbiota in deep pockets (greater than 6 mm) after subgingival scaling in 16 patients. After S/RP, the patients were divided into two groups. Group A received no further oral hygiene instructions or feedback of their

hygiene. After 16 weeks, the group returned bi-weekly for a prophylaxis and rinsed with chlorhexidine daily. Group A was examined 18, 20, 28 and 32 weeks after baseline. Group B received bi-weekly prophylaxis and oral hygiene instruction with daily chlorhexidine rinses. They were re-evaluated 2, 4, 8, 12, 16, 28, 32 weeks after baseline. Sites with excellent hygiene had a vast improvement in clinical parameters such as probing depths decreased from 6.8 mm to 4.2 mm. It was noted that there was also a retained reduction in the quantity of motile rods and spirochetes. Deeper probing depths greater than 8 mm did not improve significantly. In the Group A where the presence of supragingival plaque was uncontrolled, large numbers of spirochetes and motile rods were reestablished by 4-8 weeks. Group B, the deep sites which were kept free from supragingival plaque noted that a large proportion of motile bacteria soon recurred. The authors concluded that **S**/RP decreases the number of spirochetes with good oral hygiene, but returned to baseline levels by 16 weeks without oral hygiene [107].

I.6.4.2.2 Ultrasonic Instrumentation

In 1991, Chiew *et al* assessed the effectiveness of ultrasonic debridement of obvious calculus deposits. They were interested in noting changes in bacterial products, specifically LPS, in 34 incisors. Ten were not cleaned to serve as a control while the remaining 24 teeth were debrided with a Cavitron. The root surface was then tested for LPS. The amount of LPS from the experimental teeth ranged from less than 0.08 up to 22.39 ng compared to 1,900-29,200 ng from the controls. This supported the conclusion that ultrasonic debridement is effective in removing LPS that is concentrated on the superficial surface of a root. It supports the theory that the plaque, not the calculus, is the

pathogenic factor since various amounts of calculus remained on the instrumented root surfaces in conjunction with low levels of remaining LPS [108].

In comparing ultrasonic to sonic scalers, Baehni *et al* approached the question with an *in vitro* and *in vivo* method. In the *in vitro* study, the authors submerged 27 plaque samples which consisted of approximately 30-60% spirochetes and motile rods in saline. The samples were then subjected to ultrasonic (28,500 Hertz.) or sonic (<10,000 Hertz) vibration for 10, 30 and 60 sec. The samples were then examined with darkfield microscopy and cultured to determine the number of colony forming units per plate. The results indicated a decrease in amount of spirochetes and motile rods with either instrument which was directly proportional to time of exposure; however, the ultrasonic produced significantly greater declines. Oddly, the total number of cultivated bacteria *increased* significantly following either treatment. In the *in vitro* study, 66 periodontal pockets with 25% alveolar bone loss and probing depths greater than 4 mm were sampled for plaque composition. The sites were then treated for 10 and 30 seconds. A second plaque sample was taken and analyzed. Another time dependent decrease in spirochetes and motile rods was observed with both instruments without significant difference between the treatments. An 87-89% reduction in the number of CFU's was noted after 30 seconds of either instrumentation. The authors concluded that in regards to reducing the number of spirochetes and motile rods, ultrasonic and sonic scalers are comparable [109].

Mousques and colleagues studied the effect of a single session of S/RP on the subgingival flora in 14 patients with darkfield microscopy. Prior to treatment, one site was selected and assessed for Gingival Index, Plaque Index, probing depths, and

distribution of coccoid cells, spirochetes, and motile cells. The patient then received a single, full mouth S/RP and another site was re-evaluated in each subject. The authors used a different site for every time the patient came in for a re-evaluation which were days: 3, 7, 14, 21, 28, 35, 42, 49, 56, 70 and 90. Some trends were noted: PlI and GI scores decreased the first 2 weeks, but returned to baseline around the 3rd or 4th week. The GI and PlI declined again around the 5th to 6th week, but returned to baseline levels until the end of the experiment. In regards to probing depths, they dropped below baseline levels during the first week and with the exception of the 4th week; they remained below baseline levels until the end of the experiment. It was noted that the amount of coccoid cells at baseline (25% of total composition) increased up to 75% by the third day, but returned to initial percentages by the 3rd week. Spirochetes decreased dramatically, but returned to preliminary percentages by the 42nd day. Motile cells only decreased the first three days (from 14.8% to 3.8%), but returned to baseline by the end of the 1st week. The authors noted that the proportion of coccoid cells was negatively correlated to GI and PlI scores. However, a positive correlation was found between GI, PlI, probing depths, and the percentage of spirochetes [110].

In 1986, Gilman and Mazey evaluated the effect of the Cavitron and Prophy-jet and their ability to detoxify the root surface. They quantified the difference by comparing the amount of attachment of human gingival fibroblasts to treated and untreated root surfaces. 6 teeth were sectioned. 4 sections remained as controls, 4 were instrumented with the Cavitron, and 4 were instrumented with the Cavitron and the Prophy-jet. The sections were placed in Linbro tissue cultures to allow gingival

fibroblasts to attach to the surface. As expected, no growth was noted on calculus laden specimens. While the Cavitron samples had some mild growth, the Prophy-jet/Cavitron specimen was superior in regards to the amount of viable, attached fibroblasts [111].

I.6.4.2.3 Bacterial Repopulation after Therapy

It is understood that hand or mechanical instrumentation will change the composition of the microflora. However, these changes appear to be only temporary and the studies vary when the microflora returns to baseline concentrations. Magnusson reported that in the absence of oral hygiene, the spirochetes and motile rods were reestablished in 4 to 8 weeks [107]. Mousques observed that after a single session of S/RP, without proper oral hygiene, there was a return to baseline values by 3 months [110]. In a study of 12 patients with moderate probing depths (4-6 mm), Tabita *et al* using a split mouth design instigating one of following treatment modalities after a full mouth S/RP: one quadrant received daily professional, supragingival prophylaxis; the other was maintained only by the patient's oral hygiene. The authors noted the development of subgingival plaque within 14 days, even with daily professional care [112]. Although studies are not completely certain why the rebound occurs, the fact that the inability to remove 100% of subgingival plaque and calculus as well as lack of control on supragingival plaque removal are certain to play a role.

I.6.5 Osseous Defect Repair

Isidor and coworkers compared the effect of root planing as compared to that of modified Widman flap surgery after initial therapy in seventeen patients with advanced periodontal disease. After re-assessment, two quadrants were treated with a modified

Widman flap surgery, one with a reverse bevel flap surgery, and the final with scaling and root planing alone. The periodontal status of each patient was assessed at 3 and 6 months after bi-weekly recalls for professional prophylaxis following treatment. Although clinical gain of attachment was obtained following all three modalities, root planing resulted in slightly more gain of attachment. However, in angular osseous defects, surgery resulted in 0.5 mm coronal growth of bone while no changes were noted after scaling and root planing [113]. Renvert *et al* reported similar results when they noted virtually no bone fill after root planing after treatment by root planing [114].

I.6.6 Endotoxin Elimination

Several studies looked to see what could be done to reduce or eliminate the amount of endotoxin on the root surface. Jones and O'Leary conducted an *in vivo* clinical study. 296 root surfaces were divided in 5 sample groups. Group 1: root planed *in vivo*, until the root felt hard, smooth, and glass-like. Group 2: root planed supragingivally. Group 3: Periodontally involved teeth with no treatment. Group 4: Periodontally involved root surfaces were extracted and scaled *in vitro*. Group 5: Surgically removed, impacted 3rd molars to simulate a normal, healthy root surface. 18% of the subgingival root-planed surfaces were found to have remaining calculus. 14% of the supragingival root-planed surfaces still had calculus. In both cases, the areas where the residual calculus was found were: 1) at CEJ, 2) in root flutes and 3) at line angles. Scaling alone resulted in endotoxin values considerably greater than the values for healthy root surfaces (1.5 vs 0.25 ng respectively). However, the root-planed samples contained only about 1ng more of endotoxin than did the healthy root surfaces (0.25 ng)

[115]. The cumulative works of Nakib *et al* and Hughes and Smales, found that endotoxin only weakly adhered to the cementum [116, 117]. Moore *et al* found that 39% of the LPS could be removed by gently washing in water for 1 min and an additional 60% could be removed by brushing for 1 min with a slowly rotating bristle brush. They suggest that effective root surface debridement may be achieved by gentler methods other traditional hand instrumentation [118]. However, Nishimini and O'Leary investigated the difference of endotoxin removal of ultrasonic instrumentation compared to S/RP. They noted that S/RP was superior to ultrasonic instrumentation alone (2.09 ng. vs. 16.8 ng.) [119].

I.6.7 Single vs. Repeated Instrumentation

When comparing the results of single to repeated episodes of ultrasonic debridement, Badersten *et al* noted that a single session of 4.9 hours was comparable to a total of 7.9 hours over 3 sessions. Badersten reported that the probing depths decrease by a mean of approximately 2 mm after 9 months. A loss of attachment of 1.5 mm was noted in probing depths initially less than 3 mm, but a gain of 1.5 mm of attachment was noted in deep pockets which also were more prone to bleeding on probing. The authors concluded that in non-molars and with excellent hygiene, a single episode of ultrasonic scaling was as effective as three episodes of treatment done every 3 months [120]. A similar finding was found for molars by Caton *et al* [121]. However, Magnusson *et al* were able to reduce probing depths by 1.2 mm by 16 weeks, but reduced it another millimeter after a second instrumentation [107]. Similar results were found by Torfason *et al* whom noted a 2 mm decreased in probing depths after the first four weeks, but a second S/RP decreased probing depths by another millimeter after the 8th week. This study also found no

differences between using hand instrumentation or ultrasonic scalers in dentition with 4-6 mm probing depths except in time of treatment favoring ultrasonic scalers [122].

I.6.8 Histological Attachment after Therapy

Caton *et al* using a primate model determined that periodic S/RP, soft tissue curettage, and oral hygiene three times a week resulted in the formation of a long junctional epithelium with no new connective tissue attachment [123]. Waerhaug *et al* found a similar result with the dento-epithelial attachment being renewed within 2 weeks and suggested pocket elimination surgery for probing depths greater than 3 mm[124]. Aukhil *et al* concluded from a canine study that plaque control, not the type of attachment (long junctional epithelium or connective tissue), was the most critical factor in halting disease progression [125]. This was illustrated by Nyman *et al* in a surgical study where patients underwent periodontal surgery with inadequate oral hygiene. After following the patients for two years post-operatively, they noted a significant loss of attachment and reoccurrence of periodontal inflammation [126].

I.6.9 Periodontal Healing Time after Therapy

Proye and coworkers reported on the response of 128 periodontal pockets (3-7 mm) after a single episode of root planing in 10 patients. The patients were recalled weekly for 4 weeks for measurements, supragingival prophylaxis, and oral hygiene instruction. They noted significant improvement in gingival indices after 1 week as well as a reduction in probing depths due to recession. No additional reduction in gingival indices was noted; however, a second reduction in probing depths noted as a gain in attachment as well as the absence of bleeding on probing was noted after 3 weeks [127].

A follow up study saw no further gain during the next 3 months, which lead the authors to conclude that the a positive effects from a single episode of subgingival root planing with improved oral hygiene can be maintained every 3 months [121]. Morrison *et al* concluded that healing took at least 4 weeks [101].

In a two part, two year analysis of 82 patients with moderate to advanced periodontitis, Kaldahl *et al* evaluated four separate treatment approaches. Each patient had the molars of a quadrant assigned to osseous surgery, modified Widman flap surgery, S/RP, or supragingival scaling. In regards to probing depth reduction and gain in clinical attachment, osseous surgery was best, followed by the modified Widman flap, S/RP, and supragingival scaling showing the least improvement. The authors demonstrated that over the course of a year, the periodontium continued to repair, but the greatest changes in probing depth reduction and gain of clinical attachment can be recorded after 4 to 6 weeks [128, 129].

I.6.10 Hand Instruments vs. Ultrasonic Instruments

In a scanning electron microscope study by Meyer *et al*, manual root planing resulted in a smoother root surfaces than ultrasonic debridement [130]. However, Waerhaug demonstrated that a junctional epithelium would develop on a rough root in the absence of "bacteria or their toxins" [69]. Other noted advantages to using the ultrasonic are the potential to reduce fatigue and treatment time [97, 122, 131], alteration of the plaque, and a bactericidal effect on spirochetes [132, 133].

I.6.11 Longitudinal Studies of Nonsurgical vs. Surgical Therapy

The longitudinal studies are a complementary array that is typically categorized by geographic location. The Michigan studies (Ramfjord and colleagues) were the first to compare nonsurgical to surgical therapy. Philstrom in Minnesota, Kaldahl in Nebraska, and the Loma Linda studies of Badersten, Egelberg, and colleagues followed in university studies. The Arizona study was completed in a private practice setting by Becker. These types of studies also were done by Lindhe and Rosling in Sweden and Isidor in Denmark. After numerous appointments focused on: measurements, statistical analysis, oral hygiene instructions, and instrumentation with or without surgical access; a few generalities can be made. The primary goal must be control of the host response to plaque. Without proper compliance or maintenance, a sulcus with plaque will breakdown. If a probing depth is 3 mm or less, it can be maintained with non-surgical therapy, but can have propensity to lose attachment if instrumented too vigorously [134]. If the probing depth is greater than 4 mm, it is at greater risk to break down further periodontally in a directly proportional depth dependent trend without surgical intervention [134]. Deep probing depths heal initially the first few weeks by recession and will continue to decrease slightly as there is a gain of attachment for the next few months. Molars, especially with furcation involvement are harder to maintain and no method of access or instrumentation results in 100% removal of calculus. Additionally, there is no clinical parameter that can accurately predict further attachment loss.

I.6.12 Factors Limiting the Success of Non-surgical Therapy

I.6.12.1 Length of Therapy

The excellent results noted in the previous studies required extensive root instrumentation. In the Ramfjord study, non-surgical therapy consisted of treatment rendered by a hygienist for 5 to 8 hours, followed by an additional 6 hours by a periodontist [135]. The patients then were recalled for prophylaxis once a week for 4 weeks, and then every 3 months for maintenance. The studies by Lindhe andPihlstrom were similar in the amount of time needed [136, 137]. Many of the studies averaged nearly 10 minutes of instrumentation per tooth [134]. The Arizona study by Becker applied the same principles of the university studies and found similar results with 3 month recalls, which is promising to clinicians that this can be practically applied to private practice setting [138].

I.6.12.2 Skill Level of Therapist

Several studies such as Brayer *et al* concluded that the experience level of the operators in a study is crucial to interpreting the results. In 114 periodontally hopeless, single rooted teeth were treated by either open or closed access by a resident or periodontist operator. In shallow sites, both operators were effective in either open or closed access. However, as the probing depth deepened, open debridement proved to be more effective, especially by a periodontist. They also concluded that for periodontal pockets greater than 4 mm, open flap debridement is more effective than closed debridement. The effectiveness of S/RP is related to the operator's experience [139].

I.6.12.3 Patient Compliance

During an 8 year period, Wilson *et al* reported that in a periodontal private practice, only 16% of the treated patients were good compilers; 49% were erratic; and 34% were poor compilers [140] In a follow up study, with reminders, the number of good compilers increased to 32% [141]. Wilson states that if a patient does not deem the chronic problem as life threatening, the doctor-patient relationship will deteriorate quickly [142]. However, patients tend to comply better when they are well- informed and receive positive reinforcement [142].

I.6.12.4 Maintenance

Matuliene *et al* reported on the results of 172 periodontal patients with a mean of 11.3 years of periodontal therapy. They focused on trying to use residual probing pocket depth as a predictive parameter for periodontal disease progression and tooth loss. A probing depth less than 3 mm had an odds ratio of 5.8 for disease progression. A 5 mm probing depth had a 7.7 odds ratio. However, a 6 mm probing depth increased the odds ratio to 11 which increased to 64.2 when the probing depth was 7 mm. They concluded that sites with a probing depth of 6 mm or greater and greater than 30% of full mouth sites with bleeding on probing represented an increased risk of tooth loss [143]. A follow up study by Salvi *et al* using the same data wanted to see what the risk factors for multi-rooted teeth were. There was a significant risk for the molar to be lost if the molar presented with 2 or 3 furcations exposed prior to therapy compared to molars without furcation involvement. Smokers were significantly more at risk to lose a molar, especially if they were noncompliant with regular periodontal maintenance visits [144].

I.7 A Brief on Guided Tissue Regeneration

Procedures limited to treating the periodontal pocket such as scaling and root planing would not be expected to greatly influence new bone formation, but hopefully healing with a connective tissue attachment rather than a long junctional epithelium [145]. All available histological evidence to date demonstrates healing by a long junctional epithelium with no or minimal connective tissue attachment [146]. Although a long junctional epithelium shows equal resistance to disease as normal junctional epithelium or connective tissue, epithelial proliferation apically along the healing root surface has been shown to interfere with the establishment of a new connective tissue attachment [147]. For this reason, various techniques have been employed in the treatment of periodontal defects.

In 1976, Melcher described guided-tissue regeneration with the goal of allowing only cells from the bone, connective tissue, and periodontal ligament to repopulate the root surface before epithelial cells contacted the healing site by the use of a membrane [66]. To date, most intrabony defects are treated with full-thickness buccal and lingual mucoperiosteal flap reflection, debridement of the defect, root preparation followed by grafting of the defect with bone or a bone substitute, and a barrier membrane [148]. However, due to the inability to create a perfect seal with the membrane, epithelium can proliferate apically along a root surface. The search has been for a more effective method to exclude epithelium long enough for connective tissue or bone to grow.

I.8 Lasers and the Periodontium

Light Amplification by Stimulated Emission of Radiation or LASER is a monochromatic light that is collimated (or filtered into parallel beams) and travels along in wavelengths with amplitude. The amplitude refers to the height of a wave and is an indication of the intensity of the wave, or amount of work the beam can do. The wavelength is measured from the distance of two successive crests of a wave. It is usually quantified in microns or nanometers and is the decisive characteristic that dictates how the wave will interact with tissue. Short wavelengths (less than 350 nm) are considered ionizing and can cause DNA mutation. All lasers used in dentistry are considered non-ionizing which cause a photothermal effect on the tissue, a phenomenon from converting light energy to heat [149].

It is critical to know the emission mode (continuous or pulsed), the power density, and duration of exposure to prevent inadvertent tissue damage. Between 37°C and 49°C, the tissue experiences hyperthermia without lasting damage [149]. Over 50°C, non-sporulating bacteria become inactivated [150]. Over 60°C, coagulation and protein denaturation occurs [151]. Between 70-80°C, one can "tissue weld" for hemostasis and wound closure, but vaporization of the tissue occurs at 100 °C allowing for ablation [152]. Any temperature over 200°C leads to carbonization of the tissue. Once this occurs, it is important to remember that carbon absorbs all wavelengths and will not dissipate heat as quickly. This can rapidly lead to unwanted damage of adjacent tissue [149]. To prevent this, a clinician must understand a few basic concepts and standard laser terminology prior to use.

I.8.1 Laser Terminology

A clinician has the ability to make several adjustments to the laser except for altering the wavelength itself. Energy is expressed in joules. A joule delivered for 1 second is a watt which is the unit of power expressed by the laser. A laser has the capability to emit its energy either as a continuous beam or in pulses. The number of pulses per second is termed hertz. Average power is the amount of power interacting with tissue over a period of time. For example, if the laser is in continuous mode, this is equivalent to the power. However, if it is in a pulsed mode, it is the output power divided by the percentage of time the laser is emitting. The average power can also be calculated by energy per pulse multiplied by the hertz.

Some lasers have an articulating arm without a contact tip. These lasers have specific diameter or focal point where energy output is the greatest and therefore most effective. Other lasers have a contact tip that is usually an optical fiber to deliver the beam. The beam diameter refers to the actual size of the target spot on the tissue. This is adjusted or directed by lenses within the laser equipment.

I.8.2 Differentiation of Wavelengths and General Uses

Lasers can affect tissues in four different ways: reflection, scattering, transmission, and absorption [149]. The two most important ones are transmission and adsorption. Transmission is the laser energy passing through the tissue. Absorption is the primary and beneficial effect since each wavelength has an explicit effect on different tissue types. For example, some wavelengths are absorbed by the chromophores of blood or pigments such as dyes while others are absorbed by water or by hard tissues such as

bone. This segregates the lasers into soft tissue vs. soft/hard tissue laser groups. Another important categorization to remember is that lasers are typically named for the material of active medium such as a gas or crystal used. Soft tissue lasers include the potassium titanyl phosphate (KTP), diode, and neodymium-doped yttrium aluminum garnet (Nd: YAG) which are typically absorbed by melanin or hemoglobulin. KTP lasers have a wavelength of 532 nm [153]. Diodes usually range between 810-980 nm wavelengths while the Nd: YAG is about 1,064 nm. Soft/hard tissue lasers are absorbed by water or hydroxyapatite. The list include the erbium, chromium-doped: yttrium, scandium, gallium, and garnet (Er, Cr: YSGG) with a wavelength of 2,790 nm. The erbium-doped yttrium aluminum garnet (Er: YAG) has a wavelength of 2,940 nm. Finally, the carbon dioxide (CO_2) laser has a wavelength of 10,600 nm. Although there a many applications for currently marketed lasers, this review will primarily focus on the application of dental lasers to the treatment of chronic periodontitis.

I.8.3 Lasers and Treatment of Periodontitis

Considering the current theory for plaque-induced periodontal diseases, the keystone is the microbial component's influence on the host. It seems reasonable that laser irradiation with its bactericidal effect could be an alternative or adjunct to traditional nonsurgical therapy.

I.8.3.1 Laser vs. Debridement

Since its inception, the health care field has attempted to use the laser's beneficial properties to aid in healing. The laser was first applied *in vivo* to human teeth in 1965 [154]. A study by Tomasi *et al*, the Er: YAG laser was compared to ultrasonic debridement.

Although there was a significant difference in favor of the Er: YAG in regards to probing depth reduction and gain in attachment, it was short lived. No difference was found after four months. Tomasi also looked at bacterial samples from baseline, 2 days, and 30 days after and found no difference as well. Other than slightly less patient discomfort, the authors concluded the Er: YAG did not offer any additional advantage to subgingival debridement [155]. After a multitude of studies and reviews, the ADA's Council on Scientific Affairs stated that adjunctive laser curettage compared to mechanical or chemical curettage alone was inconclusive in 2009. This can be supported by multiple studies including Soo's findings in 2012 that compared Er: YAG laser alone versus mechanical debridement in a randomized controlled clinic study. The results concluded that mechanical debridement performed statistically better in clinical parameters such as reduction of probing depths and bleeding on probing and gain in clinical attachment at twelve weeks [156]. On the other hand, Krohn-Dale *et al* found that in 15 smokers, when two quadrants received either S/RP or pocket debridement with a Er:YAG laser, at no time point over 12 months was there any significant difference between treatments. This included mean probing depth reduction and subgingival microbiological composition, so as a result, concluded that "the results failed to support that an Er: YAG laser may be superior to conventional debridement in the treatment of smokers with recurring chronic inflammation" [157]. The question was then posed, if it cannot replace convention therapy, can it aid it?

I.8.3.2 Laser as an Adjunct to Debridement

The use of laser therapy as an adjunct to scaling and root planning is controversial and studies show equivocal results; however, there are many studies demonstrating the efficacy of the CO_2 and Nd: YAG lasers [158-163]. As the literature grows, several articles add basis to each claim against or for lasers' use as an adjunctive therapy. Ambrosini *et al* found no additional benefit of adjunctive use of a laser in a split mouth, randomized clinical controlled trial of 30 patients [164].

A similar study by Lopes *et al* had a split mouth design looking at quadrants assigned to one of four different therapies: SRP, SRP with laser, laser, and no treatment. The study of 21 patients with probing depths ranging from 5-9 mm saw an improvement of the three treatments over the non-treatment quadrant, but only a significant gain in attachment was noted in the SRP quadrant [165]. However, SRP plus laser and laser alone had a significant reduction in the percentage of sites with bacteria 6 and 12 months later [166]. Caruso *et al* looked at using a diode laser as an adjunct. In 13 patients, they treated and sampled for 8 periodontal pathogens by PCR analysis. They concluded that the diode laser may lead to a slight improvement of clinical parameters, but no significant reduction of periodontal pathogens were found in either group [167].

However, a 12 month clinical study by Kelbauskiene *et al* reported statistically significant reduction of the probing depth and gain of clinical attachment level in comparing the adjunctive use of the Er, CR: YSGG laser to scaling and root planing to S/RP alone [168].

Extensive reviews of the data by such organizations as the American Academy of Periodontology has lead to a conclusion set forth by Cobb which indicates that "there is limited evidence suggesting that lasers used in an adjunctive capacity to scaling and root planing may provide some additional benefit" [146]. Another systematic review by Karlsson *et al* also concluded that more clinical trials are needed as the current literature did not provide consistent evidence to support the treatment of chronic periodontitis with non-surgical periodontal treatment and adjunctive laser therapy [169].

I.8.4 A Brief on Carbon Dioxide Laser Studies

Although each laser's wavelength has its appropriate advantages and disadvantages, the carbon dioxide laser is able to excise and coagulate soft tissues while the wound delays epithelial migration. According to Israel, the delayed epithelialization from carbon dioxide laser wounds results from a combination of events: (1) the laser wound margins show thermal necrosis and formation of a firm eschar that impedes epithelial migration [170]; (2) the decrease in wound contraction as a result of fewer myofibroblasts, compared to scalpel wounds, leaves a greater surface area remaining to be epithelialized [171]; (3) the thin layer of denatured collagen found on the surface of the laser wound acts as an impermeable dressing in the immediate postoperative period, which reduces the degree of tissue irritation from oral contents [172]; and (4) reduced inflammation in the laser-induced wound can provide less stimulus for epithelial migration [171 173]. Rossmann *et al* and Centty *et al* have shown the carbon dioxide laser can effectively remove gingival epithelium without causing damage to the underlying connective tissue [174, 175]. A follow-up animal study by Rossmann *et al* concluded that the

de-epithelialization by a carbon dioxide laser impeded epithelial down-growth following periodontal surgery for up to 14 days longer than conventional flap techniques [176]. In 1998, Israel *et al* verified with human histology the ability to obtain clinical new attachment on a previously diseased root surface using the CO_2 laser [173]. A similar human histologic study from Yukna in 2007 used the Laser-Assisted New Attachment Procedure (LANAP). The study compared teeth that were scaled and root planed versus teeth that were scaled and root planed with the addition of Nd:YAG treatment of the sulcus. The findings reported that the LANAP-treated specimens showed new attachment while the majority of the control teeth had a long junctional epithelium [145]. This is supported by another human histological study of the LANAP technique by Nevins *et al* [177].

With the use of a low-powered pulsed Nd:YAG laser, a study evaluated the possibility of periodontal pathogen reduction during sulcular debridement. The results concluded that the adjunctive therapy of the laser may have altered the microflora of the sulcus. Neill *et al* concluded that the adjunctive use of the Nd:YAG laser may provide an advantage over scaling and root planing alone [178].

In 2014, Dilsiz *et al* reported on the use of a potassium-titanyl-phosphate (KTP) laser as an adjunct to traditional therapy. In a split mouth design of 24 patients, clinical parameters were taken at baseline, 2 months and 12 months post-operative. Both groups noted significant reductions in bleeding on probing and probing depths with gains in attachment. However, the test group (KTP + SRP) had significantly better clinical parameters than conventional therapy alone [179].

Kojima *et al* reported that use of a carbon dioxide laser killed more than 99% of *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* at energy densities of 7.5 and 12.5 J/cm^2 and energy densities of more than 7.5 J/cm^2 significantly decreased the biological activity of LPS. This was done *in vitro* with the use of a standard handpiece that delivered a defocused beam of approximately 5 mm at the tip [180]. Numerous studies have been done to see the effect of a CO_2 beam on a root surface. Israel, Fayad, Barone, and Anic all found that the thermal effects of a CO_2 laser lead to charring or melting of the root surface which could contribute to the absence of PDL attachment [181-185]. However, Pant *et al* found treated hopeless root surfaces lased with a CO_2 laser (from 5 cm at 3 W for 0.8, 1.0 and 1.2 s), compared to other root surfaces treated with either tetracycline hydrochloride (2.5%), citric acid (saturated solution, pH 1), hydrogen peroxide (6%), or EDTA (5%; pH 7.4) for 3 min [183]. They concluded that that CO_2 laser irradiation for 1.0 s may promote comparatively better attachment of periodontal ligament fibroblast on dentinal root surfaces than the conventional chemical conditioning agents used in the study [183].

The proposed study will be using a CO_2 laser with an ablative prototype handpiece and hollow tips (Spectra Lasers Ltd, Denver, CO) that allows for the laser beam to be focused directly into the sulcus and away from the root surface. The treatment protocol will involve the laser debridement procedure to be performed every 10 days for three appointments, following scaling and root planing at a setting of 8 W, continuous mode. The complete sweep of the handpiece into the periodontal pocket is about 2 seconds total. This is in concurrence with the protocol used by Kelbauskiene *et*

al which typically required an average of three appointments. Kelbauskien *et al* performed the procedure once a week for each millimeter of pocket reduction needed to obtain a normal probing depth of 3 mm or less [168].

I.9 Aim

This will be a prospective, randomized controlled clinical trial comparing the clinical outcome of using the carbon dioxide laser decontamination technique in conjunction with scaling and root planing (test sites) versus scaling and root planing alone (control sites) for the treatment of chronic moderate to severe periodontitis. The purpose of this study is to compare these techniques, specifically the additional benefit of laser decontamination in clinical parameters and bacteriologic sampling.

II. LASER ASSISTED NONSURGICAL THERAPY

II.1 Synopsis

II.1.1 Background

During the treatment of moderate to severe chronic periodontitis, non-surgical therapy typically leads to a gain in clinical attachment; however, it is most likely due to the formation of a long junctional epithelium. As the technologies of lasers have improved, the search for an application to improve clinical parameters of chronic periodontitis has grown exponentially.

II.1.2 Methods

This report presents a novel approach to the treatment of moderate to severe, chronic periodontitis utilizing the carbon dioxide (CO_2) laser in combination with scaling and root planing. This study presents the clinical and bacterial PCR findings of 14 patients that were compared in a split-mouth design and followed for 6 months. To the authors' knowledge, this is the first reported case series utilizing the CO_2 laser for sulcular decontamination in combination with scaling and root planing for the treatment of chronic periodontitis via non-surgical therapy.

II.1.3 Results

There was a significant change in all clinical parameters from baseline to the 3 month mark in both the control (S/RP) and test sites (LANST). However, there was no difference noted for any of the clinical parameters measured between the test and control sites between the 3 and 6 month time points.

II.1.4 Conclusion

Sites treated with the LANST procedure tended to show a greater decrease in probing depths, greater gains in clinical attachment levels, and bacterial levels; however, the results were not statistically significantly better than scaling and root planing alone.

II.2 Introduction

The foundation of any periodontal procedure begins with the removal of sub- and supragingival plaque and calculus to allow healing to occur in the periodontium. As the literature continuously searches for methods to improve the periodontal environment, it is always stressed that a clinician and patient must be able to avert the primary etiology of plaque. In recent years, lasers have entered the dental realm in attempts to use their unique properties to possibly improve on such "tried and true" methods.

One such a benefit may be the CO_2 laser's ability to decontaminate the periodontal environment. Kojima *et al* reported that use of a CO_2 laser at energy densities of 7.5 and 12.5 J/cm^2 killed more than 99% of *Porphyromonas gingivalis* and *Actinobacillus actinomycetecomitans* and energy densities of more than 7.5 J/cm^2 significantly decreased LPS biological activity[180]. This was done in vitro with the use of a standard handpiece that delivered a defocused beam of approximately 5 mm at the tip [180]. The proposed study will be using a CO_2 laser with an ablative prototype handpiece (Photonic Resources, Denver CO), that allows for the laser beam to be focused directly into the sulcus. The treatment protocol will involve the laser procedure to be performed every 10 days for three appointments, following scaling and root planing. This is in consensus with the Kelbauskiene *et al* protocol where an average of three appointments

was needed. Their endpoint was to have the same procedure performed weekly for each millimeter of pocket reduction desired to obtain a normal probing depth of 3 mm or less [168].

This will be a prospective, randomized controlled clinical trial study comparing the clinical outcome of using the CO_2 laser decontamination technique in conjunction with scaling and root planing (test sites) versus scaling and root planing alone (control sites) for the treatment of periodontitis. The purpose of this study is to compare these techniques, specifically the additional benefit of laser decontamination.

II.3 Materials and Methods

II.3.1 Patient Criteria

This case series consisted of fourteen patients (5 male and 9 female) ages 34-65 (mean 54 years). Study subjects were required to have a minimum of two contra-laterally similar periodontal probing depths (PD) ≥ 5mm with clinical attachment loss (CAL) ≥ 4mm on two or more teeth. Exclusion criteria included: the tooth in question is considered periodontally hopeless as set forth by McGuire's criteria [186], systemic conditions which are generally considered to be a contraindication to periodontal treatment which include but are not limited to: uncontrolled diabetes, uncontrolled hypertension, etc.. Patients that also required antibiotic coverage prior to dental procedures as defined by the 2007 American Heart Association guidelines, or have taken medication such as antibiotics, steroids, anticoagulants, or anti-inflammatory agents within three months prior to treatment were not included. Pregnant or lactating females, current smokers beyond 10 cigarettes per day, and having had scaling and root planing

within the past six months were also excluded. Patients and teeth observed in the study are shown in Table 1. Approval for research was granted by the Institutional Review Board at Texas A&M University, Baylor College of Dentistry. All subjects signed a written informed consent document prior to treatment.

II.3.2 Bacterial Sampling

After passing a screening examination, but prior to therapy, bacterial samples were taken for analysis. To test for any change in periodontal pathogens during the treatment procedure, samples were taken from the 4 deepest probing depths (2 test and 2 control sites) by sterile endodontic paper points for 30 seconds and immediately placed into a sterile micro-centrifuge tube with 0.5 mL RNALater. Samples were frozen at 5°C until further analysis via multiplex PCR for presence of known suspected periodontal pathogens by OralDNA Labs (7400 Flying Cloud Drive, Suite 150, Eden Prairie, Minnesota 55344-3720).

II.3.3 DNA Extraction

From the sample containing 2 paper points, each in 0.5 mL of RNAlater (Qiagen, Germany), DNA extraction was performed through a combination of mechanical disruption of the bacterial cell and ion-exchange column purification. The 2 paper point and RNAlater solutions were combined to 1 vial and centrifuged at 10,620 RCF for 5 minutes. Approximately 900 µL of RNAlater supernatant were aspirated off and replaced with the same volume of 0.9% saline oral rinse solution. The 1 mL of oral rinse was combined with 300 µL of zirconium beads and homogenized at 2500 rpm for 10 minutes (Tallboys High Throughput Homongenizer, Thermo-Fisher). The resulting mixture was

centrifuged to sediment the zirconium beads, and DNA in 200 µL of the liquid fraction was purified using silica membrane technology (Qiacube HT DNA extractor; Qiagen, Germany).

II.3.4 Analysis of Periodontal Bacteria

Automated PCR setup is performed using a CAS-4200 Robotic Workstation (Qiagen, Germany). Eleven bacterial species (Table 1: Bacteria Tested) are detected using asymmetric multiplexed polymerase chain reaction (PCR) with primers and molecular beacons designed to specific gene regions of each bacterial species.

Table 1: Bacteria Tested

Reaction A	Reaction B	Reaction C	Reaction D
Actinobacillus actinomycetemcomitans	*Prevotella intermedia*	*Campylobacter rectus*	*Fusobacterium nucleatum/periodonticum*
Eubacterium nodatum	*Capnocytophaga spp. (gingavalis, ochracea, sputigena)*	*Tannerella forsythia*	*Treponema denticola*
Porphyomonas gingivalis	*Peptostreptococcus micros*	*Eikenelia corrodens*	Internal Control: Apolipoprotein B

Three PCR reactions each contain primers and beacons specific for three bacterial species and the fourth reaction contains primers and beacons for two species plus a set designed to amplify the human DNA sequence ApoB. Amplification and detection were performed using a Qiagen RotorGene (Qiagen, Germany). Parameters for read cycle and probe melt temperature are optimized for each bacteria species. Fluorescent emission resulting from molecular beacon hybridization is read at the determined read cycle of the

PCR reaction and compared to the standard curve of known plasmid standards fluorescence to provide a semi-quantitative analysis of patient sample concentration for each bacterium. The calculated bacteria concentration of each species is compared to a clinical threshold concentration, determined through peer-reviewed literature research, and reported as HIGH, LOW or NOT DETECTED (ND) relative to the clinical threshold**.** The ND range is determined by the limit of detection of each batch based on the fluorescence of the blank controls (noise). In general, the ND range is ~10^3 copies/mL and below. The low range is any signal between the ND range (~10^3 c/mL) and the high value. For a bacterial load to be considered high, the concentration must be greater than the following values: AA $\geq 10^4$ c/mL, the red complex (PG, TG, TD) $\geq 10^5$ c/mL and remaining bacteria $\geq 10^6$ c/mL.

II.3.5 Clinical Parameters

One of two blinded examiners (JR or DK) made clinical measurements at each time point. Measurement of the depths of the periodontal sulcus (PD) measured with a UNC 15 periodontal probe to the nearest 1 mm increment. A single UNC 15 probe was used for all examinations. Six measurements were made around each tooth involved in the study: mesio-facial and lingual, mid-facial and lingual, and disto-facial and lingual surfaces. Recession (REC), bleeding on probing (BOP), furcation involvement (FUR), and mobility (MOB) were also recorded. There were 173 teeth in 14 patients that were included in the study. The distribution of type of tooth per patient is given in Table 2: Distribution of Tooth Type According to Patient.

Table 2: Distribution of Tooth Type According to Patient

Patient	Sex	Molar	Premolar	Incisor/Canine
1	F	6	4	6
2	F	10	8	
3	F	4	2	
4	M	4	4	2
5	M	4	8	8
6	M			12
7	F	4	2	
8	F	3	4	2
9	F	4	4	6
10	F	4	4	2
11	F	8	8	4
12	M			6
13	M	3	3	6
14	F	4	4	6
Totals		58	55	60

After measurements were completed and recorded, patients were subjected to scaling and root planing (S/RP) under local anesthesia on all sites greater than 4 mm. All S/RP sessions were typically completed in one session without time constraint by one

examiner (JDE). Immediately following S/RP, the patient's left or right side was randomly assigned to the test or control group (split-mouth design) using a coin flip to designate a treatment side. The control side did not receive any additional treatment except for a sham pass with the handpiece so as to prevent possible patient bias. To decontaminate the gingival margin, the test side was treated using a CO_2 laser beam conditioned specifically to ablate set at 4 watts continuous mode. For this study, the Azuryt CTL 1401, CO_2 laser was used (Figure 1: Azuryt CTL 1401, CO2 (North American Clinical Laser LTD, Denver, CO).

Figure 1: Azuryt CTL 1401, CO2 (North American Clinical Laser LTD, Denver, CO)

The prototype handpiece delivers a power density of approximately 280 W/cm2 through a "tip" with an internal diameter of 0.762 mm (Figure 2: Handpiece (Photonic Resources LTD, Denver, CO).

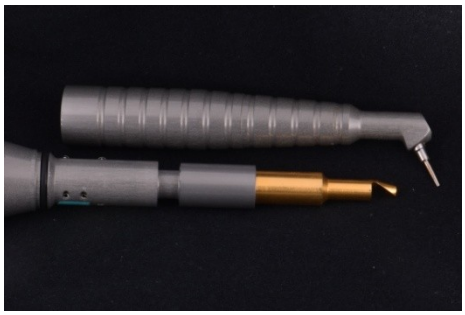


Figure 2: Handpiece (Photonic Resources LTD, Denver, CO)

The setting was then increased to 8 watts continuous mode to deliver a power density of approximately 561 W/cm^2 and the tip of the handpiece placed intrasulcularly to decontaminate and ablate the sulcus. Care was taken to avoid using the CO_2 laser on hard tissue or mucosa by maintaining a parallel orientation to the long axis of the tooth. The laser was continuously moved in the sulcus and took approximately 2 seconds to "walk" the tip from mesial to distal sites. The patients returned at 10, 20, and 30 days post-scaling for supragingival prophylaxis, oral hygiene instructions, and additional laser therapy (at the same settings) to the test side in an effort to block epithelial downgrowth on the root surface and decontaminate the sulcus, using a previously published protocol [11]. This was performed under local anesthesia as needed for patient comfort. Patients were evaluated at 3 months and 6 months post-scaling. After bacterial sampling and

measurements were taken at the 3 and 6 month appointments, the patients received a supragingival prophylaxis. Patients that presented with probing depths ≥ 5 mm at the final evaluation were referred to the graduate periodontal clinic for further treatment options.

II.3.6 Statistical Methods

Each site had the following characteristics measured at baseline, three month, and six month time points: probing depth (PD), recession (VR) [converted to clinical attachment level (CAL)], bleeding on probing (BOP), Miller mobility Scores, Furcations (Glickman), and modified O'Leary Plaque Index (PI), which is given as percent of plaque free sites. For variables measured at the three time points, a longitudinal approach for nonparametric and parametric data was used to analyze the data according to group classification and for PD greater than 5 mm, assuming an unstructured covariance matrix, and a mixed effect between time and the variable of interest. BOP was measured as percent of sites bleeding for longitudinal data analysis. Time was also treated as an ordinal variable as opposed to a continuous linear since not all patients are measured at the same time. Additionally, the model with ordinal time proved to be a better fit [observing the AIC and the -2log(likelihood)].

Results were tabulated and analyzed as described above using SAS 9.3 and R, in particular prewritten functions such as proc mixed with proc ranked to use Friedman's method, proc glimmix (for BOP), and proc univariate for all variables to test for normality. In a longitudinal study, the purpose is to test for outcome as a function of time, and to determine if there is a significant difference between treatment groups at each time

point. To test this, a test of interaction is required, followed by an analysis of the individual variables. In the analysis, at an $\alpha = .05$, interaction is tested between the variable of interest and time (baseline, 3 months, 6 months): then each individual variable is tested. Fundamentally, the following hypothesis is tested:

H_0: There is no interaction effect for the variable in consideration.

H_A: There is an interaction effect for the variable in consideration.

If this test rejects the null (i.e. p-value$< .05$ in the above hypothesis test), then the test for treatment effect is as follows:

H_0: There is a treatment effect for the variable in consideration.

H_A: There is no treatment effect for the variable in consideration.

and for time:

H_0: There is no time effect for the variable in consideration.

H_A: There is a time effect the variable in consideration.

In SAS, it is tested as a Type III test for effects, and is tested compared to an F distribution. Therefore, to reject the null hypothesis, p-value must be $< .05$. In order for there to be at least significant group difference, there must be at least a marginally significant interaction difference and group difference.

II.4 Results

II.4.1 Clinical Results

II.4.1.1 Probing Depths

In Figure 3, the overall baseline PD starts at 4.04 ±0.060 mm and decreases to 3.25 ±0.051 mm for both groups at the 3 month mark. At 6 months, there was further decrease in both groups to 3.05 ±0.044 mm. Breaking down into test versus control sites, the test sites averaged at 4.16 ±0.086 mm at baseline. In contrast, the control sites overall baseline measurement was 3.93 ±0.083 mm. From baseline to 3 months, a decrease of 0.80 ±0.053 mm was noted for all groups with the test decreasing by 0.88 ±0.076 mm compared to the control's decrease of 0.71 ±0.730 mm.

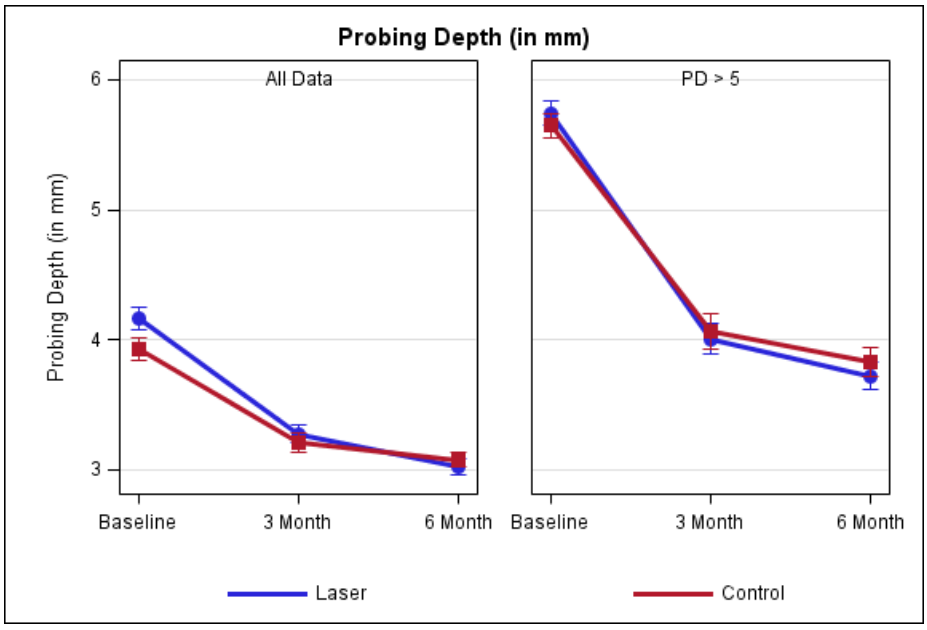


Figure 3: Probing Depth vs. Time Comparing Laser to Control Treatments

When an analysis was made for all *PD≥5* mm, the overall baseline measurement starts at 5.7 ±0.003 mm (T: 5.74 ±0.073 mm vs. C: 5.65 ±0.07 mm) and decreases to 4.04 ±0.003 mm for both groups at 3 months (T: 4.01 ±0.093 mm vs. C: 4.07 ±0.105 mm). Probing depths further decreased to 3.77 ±0.003 mm at 6 months (T: 3.72 ±0.079 mm vs. C: 3.83 ±0.085bmm). Within Figure 3, both graphs do not show much difference between trajectories and the overall values of means. The control group's probing depths, overall, are slightly deeper than the treatment group at the 3 and 6 months for *PD≥5*. There is an overall decrease of 0.99 ±0.051 mm in PD. In comparing test to control sites, the test decreased by 1.14 ±0.073 mm compared to the control's 0.85 ±0.070. When analyzing sites initially >5 mm, a 2.02 ±0.099 mm decrease was noted for the test compared to 1.42 ±0.101 mm for control sites. Overall, in sites initially ≥5 mm, a 1.93 ±0.004 mm decrease was noted. According to the data presented in Table 3 which shows the longitudinal effect of treatment group and time for corresponding to All Data to the left and PD>5 to the right of the bold divider, there is no significant difference between the treatment groups for both *All Data* and *PD≥5* over time.

Table 3: Longitudinal Data Analysis of Probing Depth

	All Data		PD >5	
	F-Value	p	F-Value	p
Group	0.670	0.4129	2.274	0.1315
Time	102.870	<0.0001	309.286	<0.0001
Group∗Time	3.112	0.2110	1.273	0.5293

II.4.1.2 Clinical Attachment Level

In Figure 4, overall (*All Data)* indicates no significant difference between the two groups, except at baseline. At baseline the CAL is 4.03 ±0.084 mm for the test group and 3.72 ±0.079 mm for the control group. Both groups improve to 3.24 ±0.053 mm at three months. The levels further improve between three months and six months by 0.24 ±0.050 mm to 3.05 ±0.046 mm. When focusing on sites with *PD≥5,* the data shows that there is a sharp decrease in CAL between 5.21 ±0.003mm baseline to 3.83 ±0.004 mm at the three month visit (3.90 ±0.105 mm for control and 3.77 ±0.103 mm for test). Between the three month and six month visit there is another decrease of 0.28 ±0.004 mm to 3.56 ±0.003 mm.

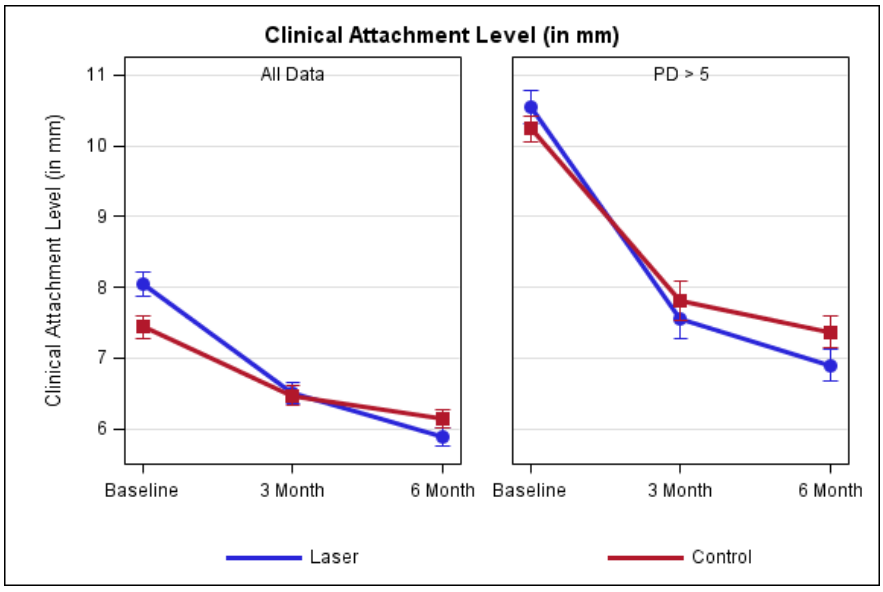


Figure 4: Clinical Attachment Level vs. Time Comparing Laser to Control Treatments

When focusing on sites with PDs ≥5 mm initially, the CAL test sites improved from baseline to 6 months by 1.83 ±0.107 mm compared to the control group's improvement of 1.44 ±0.103 mm. There is no statistical significance between the graphs. According to the data presented in the Table 4, there is a significant difference over time between the treatment groups for both *All Data* and *PD≥5*; however because the p-value for the groups is largely insignificant, it appears that this significant difference largely comes from the changes in time.

Table 4: Longitudinal Data Analysis for Clinical Attachment Level

	All Data		PD >5	
	F-Value	p	F-Value	p
Group	0.06	0.4447	0.07	0.7981
Time	125.98	<0.0001	153.85	<0.0001
Group∗Time	7.85	0.0004	3.26	0.0393

II.4.1.3 BOP

At baseline, the sites with BOP was 70.73±5.458% for control sites and 67.66±4.665% for the laser treated group. Between baseline and three months, there is an overall decrease to 31.70 ±6.453% with 35.22 ±4.599 % of control sites and 39.78 ±5.557% sites for the laser group still presenting with BOP. Both groups show an increase in BOP between 3 month and 6 month visits to 37.60 ±5.945% for control and 42.46 ±6.956% for the laser group. The percentage of sites with bleeding from 3 month and 6 month show that controls tended to have lower percentages than the test group. However, there does not appear to be any significant difference between the groups at any of the time points as seen in Figure 5: Bleeding on Probing vs. Time Comparing Laser to Control Treatments.

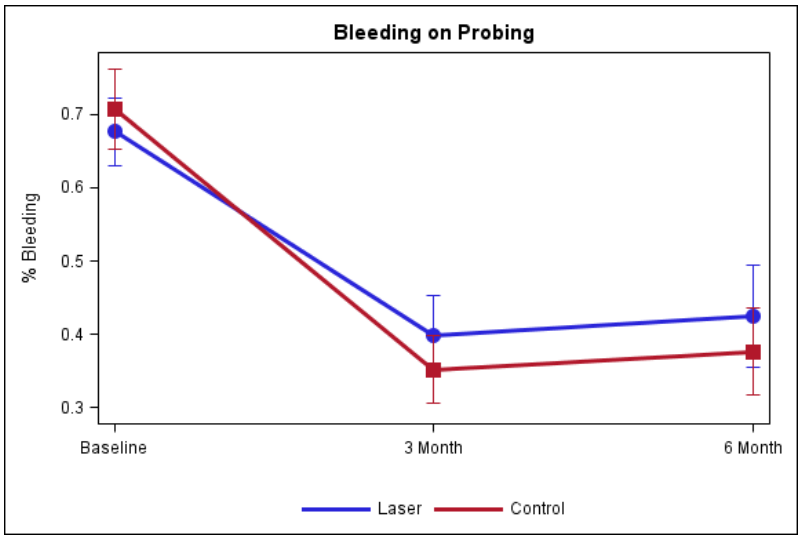


Figure 5: Bleeding on Probing vs. Time Comparing Laser to Control Treatments

According to Table 5, there is no significant difference in effect between the treatment groups globally (p-value=0.6710). This validates Figure 4's conclusion.

Table 5: Longitudinal Data Analysis for Bleeding on Probing

	***F*-value**	***p*-value**
Group	0.14	0.7158
Time	24.97	<.0001
Group∗Time	0.41	0.6710

II.4.1.4 Plaque Index

Considering Plaque Index, Figure 6 shows that there is an increase in percent of plaque free for both treatment and control groups.

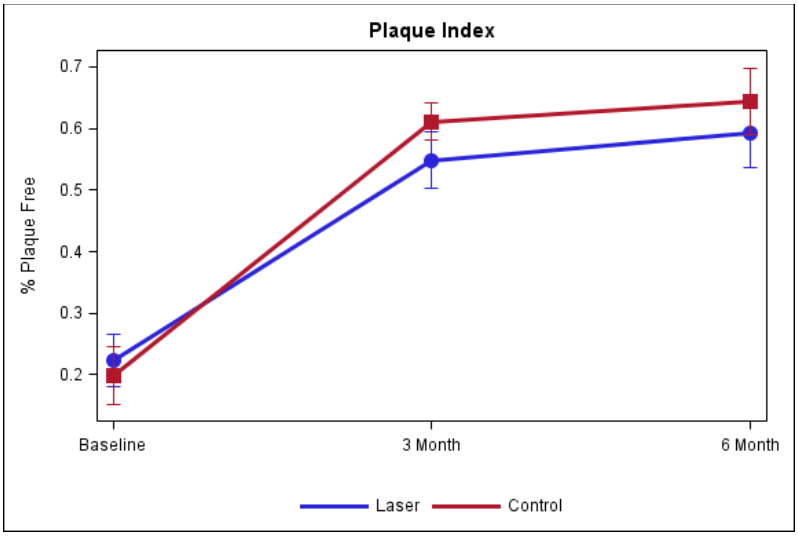


Figure 6: Plaque Index vs. Time Comparing Laser to Control Treatments

Both start at baseline of 21.08 ±4.197% plaque free and increase to 61.11 ±3.051 % plaque free for the control group and 54.85 ±4.617 % plaque free for the test group or 57.99 ±3.268 % plaque free overall. There is an increase from the three month to the six month visit by -3.87±4.333%. The control group rises to 64.38 ±5.396 % and the laser group rises to 59.33 ±5.628 % plaque free. Overall there does not appear to be a significant difference between the groups. According to Table 6, there is no significant difference in effect between the treatment groups globally (p-value=0.5579). This confirms what was concluded in Figure 5.

Table 6: Longitudinal Data Analysis of Plaque Index

	***F*-value**	***p*-value**
Group	0.44	0.5118
Time	49.58	<.0001
Group∗Time	0.60	0.5579

II.4.1.5 Mobility

Table 7 shows that there is not much difference in Miller classification at baseline: 50-65% of teeth in each group exhibited no mobility. For the control group, approximately 20% of teeth exhibited Class 1 mobility compared to 25% of teeth in the laser treatment group. At baseline, there was an equal proportion of Miller Class 2 for both treatment groups. At three months, there was increase in the proportion of teeth without mobility. There was a decrease in the proportion of Miller Class 1 and 2 for both treatment groups, and there was an appearance of a single tooth with Class 3 mobility in the laser group. There was further improvement in the percentage of teeth without mobility between the 3 month and 6 month visits for the control group compared to a slight decrease with laser treatment due to the tooth with Class III mobility. For the laser treatment group, there was an increase in the number of Class 1 between 3 and 6 months. No change was in the proportion of Class 2 and 3 mobility in the laser treated, and there was a decrease in Class 2's between three and six months. Table 8 indicates that there was no significant difference between the treatment groups and no global difference (i.e. time and group simultaneously).

Table 7: Miller Classification at Each Time Point

Control

	Baseline	3 Month	6 Month
0	34	43	44
1	22	7	7
2	8	3	2
3	0	0	0

Laser

	Baseline	3 Month	6 Month
0	29	41	38
1	16	7	10
2	8	4	4
3	0	1	1

Table 8: Ordinal Outcome Model for Longitudinal Data Analysis of Miller Classification with Respect to Time and Treatment Group

Model Member	Estimate	z-value	p-value
Test: Control	0.3378	0.70	0.4833
Month 3: Baseline	-0.9824	-3.00	0.0027
Month 6: Baseline	-1.2038	-3.18	0.0014
Test ∗Month 3: Test*Baseline	0.0572	0.13	0.8990
Test∗Month 6: Test*Baseline	0.5152	1.07	0.2856

II.4.1.6 Furcations

Table 9 shows the different furcation classes in each treatment group at each time point.

Table 9: Glickman Furcation Classification at Each Time Point

Control			
	Baseline	3 Month	6 Month
0	21	28	29
1	36	28	29
2	8	9	7
Laser			
	Baseline	3 Month	6 Month
0	17	27	30
1	38	32	28
2	10	6	7

There is an equal proportion of teeth without furcation involvement at baseline for each treatment group (approximately 45%), which decreases to just above 25%. There is than an increase to about 50% for the control group and about 40% for the treatment group from the three month to six month time points. From baseline to three months,

there is an increase in the proportion of Class 1 furcations for both treatment groups. Between three month and six month there is decrease in the proportion of Class 1 furcations. There are more Class 1 furcations in the control group compared to more Class 2 furcations in the laser treatment group at baseline. At 3 months, there was a two fold increase in the quantity of Class 2 furcations in the control group while the furcation involvement remained steady for the laser treatment group. At 6 months, there is a decrease in Class 2 furcations and an increase in the same furcation level for the control and laser treatment groups, respectively. Table 10 shows that there is no significant difference between the group and time interactions; indicating there is no significant effect with group and time globally. Time was treated as categorical to allow for an analysis of fixed effects. There is no significant difference between the group and time interactions. Table 11 represents a summation of the clinical data.

Table 10: Ordinal Outcome Model for Longitudinal Data Analysis of Furcation Level with Respect to Time and Treatment Group

Model Member	Estimate	z-value	p-value
Test: Control	0.4413	0.83	0.4065
Month 3: Baseline	-0.3919	-0.99	0.3225
Month 6: Baseline	-0.6282	-1.72	0.0861
Test∗Month 3: Test*Baseline	-0.5630	-1.01	0.3126
Test∗Month 6: Test*Baseline	-0.4967	-0.9279	0.3534

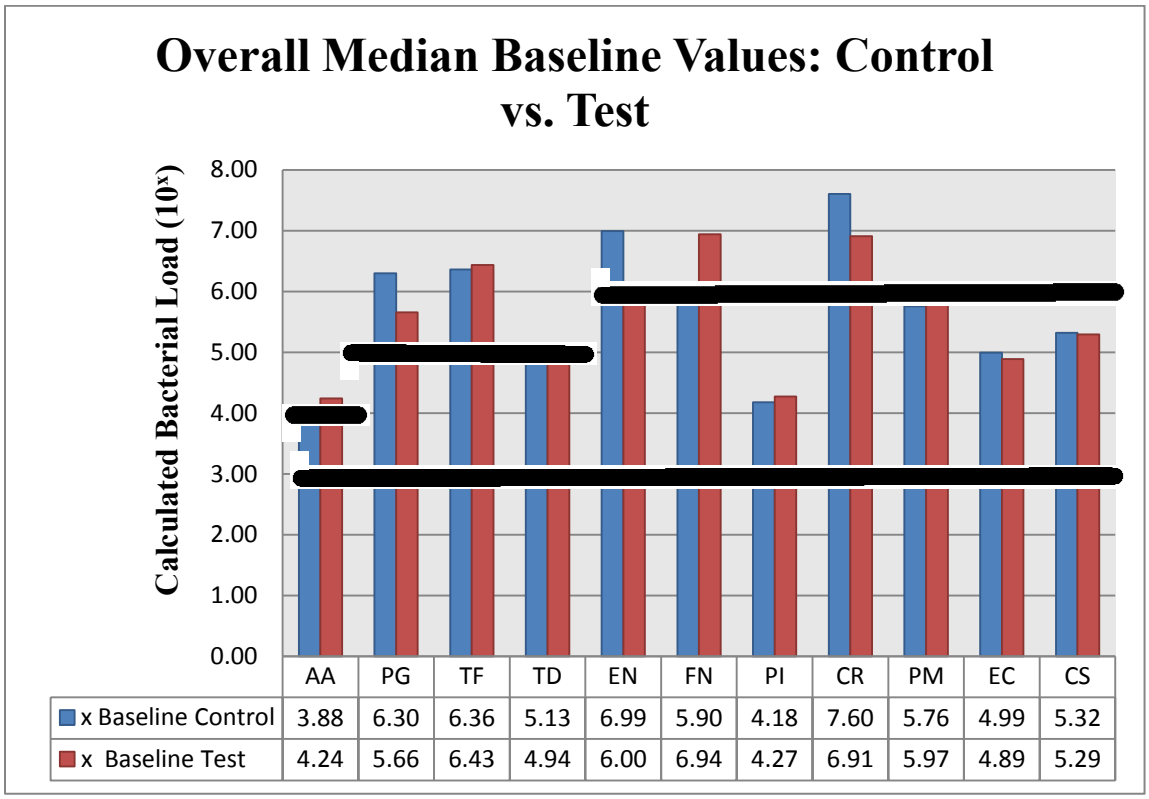
Table 11: Summary of Clinical Parameters

	All Data					PD≥5		
	PD (mm)	CAL (mm)	Recession (mm)	BOP (% bleeding)	PI (%Plaque Free)	PD (mm)	CAL (mm)	Recession (mm)
Baseline								
Test	4.16 ±0.086	4.03 ±0.084	-0.13 ±0.044	67.66±4.665	22.32 ±4.300	5.74 ±0.073	5.27 ±0.092	-0.27 ±0.052
Control	3.93 ±0.083	3.72 ±0.079	-0.21 ±0.051	70.73±5.458	19.84 ±4.726	5.65 ±0.070	5.12 ±0.070	-0.35 ±0.068
Overall	4.04 ±0.060	3.87 ±0.058	-0.17 ±0.034	69.20±4.621	21.08 ±4.197	5.70 ±0.003	5.21 ±0.003	-0.31 ±0.002
Baseline to 3 Month								
Test	0.88 ±0.076	0.77 ±0.090	-0.06 ±0.047	27.88 ±7.054	-32.54 ±6.777	1.73 ±0.102	1.50 ±0.118	-0.12 ±0.066
Control	0.71 ±0.730	0.48 ±0.078	-0.28 ±0.052	35.52 ±6.643	-41.27 ±4.893	1.58 ±0.106	1.21 ±0.104	-0.32 ±0.075
Overall	0.80 ±0.053	0.63 ±0.060	-0.17 ±0.035	31.70 ±6.453	-36.90 ±3.268	1.66 ±0.004	1.37 ±0.004	-0.22 ±0.003
3 Month								
Test	3.27 ±0.071	3.25 ±0.077	-0.07 ±0.048	39.78 ±5.557	54.85 ±4.617	4.01 ±0.093	3.77 ±0.103	-0.15 ±0.068
Control	3.21 ±0.074	3.23 ±0.740	0.06 ±0.048	35.22 ±4.599	61.11 ±3.051	4.07 ±0.105	3.90 ±0.105	-0.03 ±0.053
Overall	3.25 ±0.051	3.24 ±0.053	0.00 ±0.034	37.50 ±4.759	57.99 ±3.268	4.04 ±0.004	3.83 ±0.004	-0.09 ±0.002
From 3 Month to 6 Month								
Test	0.25 ±0.56	0.31 ±0.074	0.05 ±0.049	-2.68 ±4.786	-4.46 ±5.258	0.28 ±0.083	0.33 ±0.109	0.05 ±0.072
Control	0.14 ±0.055	0.17 ±0.068	0.03 ±0.035	-2.38 ±5.520	-3.27 ±4.788	0.24 ±0.095	0.22 ±0.107	0.03 ±0.049
Overall	0.19 ± 0.039	0.24 ±0.050	0.04 ±0.030	-2.53 ±4.940	-3.87±4.333	0.26 ±0.003	0.28 ±0.004	0.04 ±0.002
6 Month								
Test	3.02 ±0.064	2.94 ±0.064	-0.12 ±0.049	42.46 ±6.956	59.33 ±5.628	3.72 ±0.079	3.44 ±0.087	-0.20 ±0.061
Control	3.08 ±0.061	3.07 ±0.066	0.03 ±0.046	37.60 ±5.945	64.38 ±5.396	3.83 ±0.085	3.69 ±0.086	-0.06 ±0.058
Overall	3.05 ±0.044	3.05 ±0.046	-0.05 ±0.034	40.03 ±6.285	61.86 ±4.809	3.77 ±0.003	3.56 ±0.003	-0.13 ±0.002
From Baseline to 6 Month								
Test	1.14 ±0.073	1.08 ±0.080	-0.01 ±0.051	25.20 ±26.363	-37.00 ±6.509	2.02 ±0.099	1.83 ±0.107	-0.07 ±0.064
Control	0.85 ±0.070	0.65 ±0.075	-0.24 ±0.049	33.13 ±25.011	-44.54 ±6.654	1.42 ±0.101	1.44 ±0.103	-0.30 ±0.071
Overall	0.99 ±0.051	0.87 ±0.055	-0.13 ±0.035	29.17 ±24.178	-40.77 ±5.907	1.93 ±0.004	1.65 ±0.004	-0.18 ±0.002

II.4.2 Bacterial Analysis

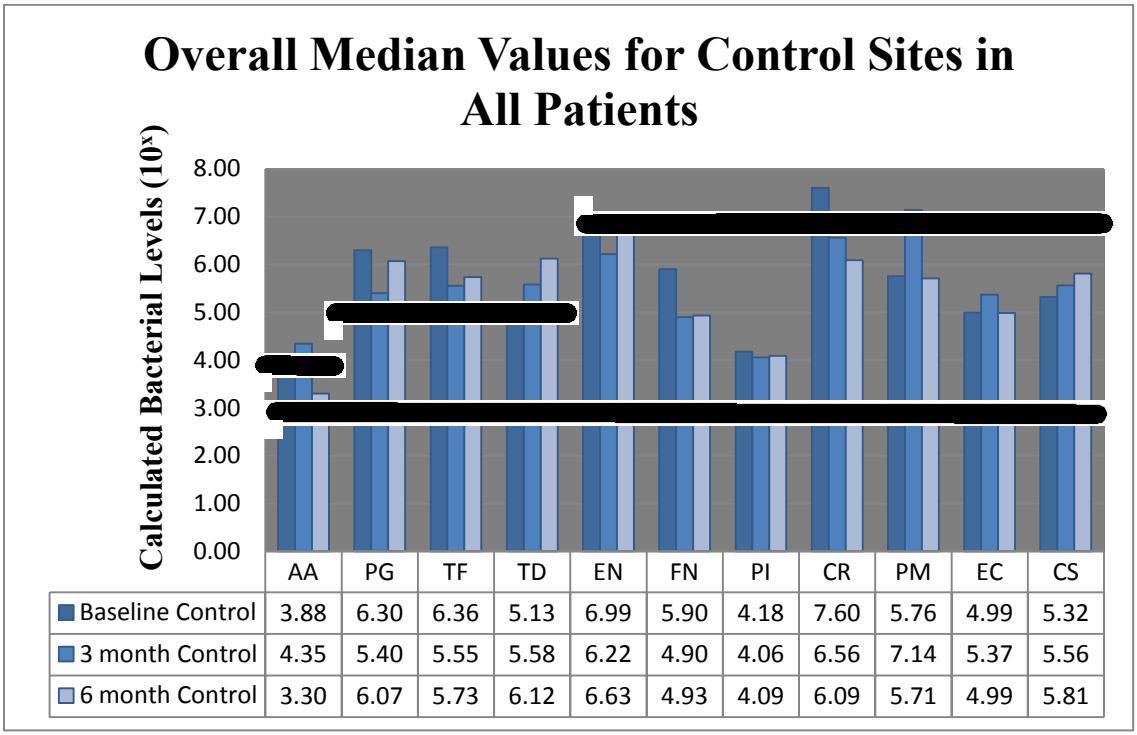
In the Figures 7, 8, and 9, several black lines are present to delineate boundaries set for a bacterial load to be considered N/D, Low, or High. Any column ending below the 3.00 line is considered N/D. Any column ending between the 3.00 line and the superior line (at 4.00, 5.00, and 6.00) is in the Low detection range. Any column ending above the line is considered to have a High bacterial load. The overall median baseline values are plotted in Figure 7 which shows that both groups present with high detectable levels of PG, TF, and CR. The control group had a higher percentage of EN compared to

the higher levels of AA and FN noted in the test group. The medians of each calculated bacterial level are plotted against the time points of interest in Figures 8 & 9 in which generalized trends can be visualized. In an attempt to simplify the data as much as possible, the bacteria will be addressed via complexes overall per treatment group. When analyzing between test and controls, Figure 10 depicts changes from Baseline to 3 month and Baseline to 6 month for both groups. The trends noted suggest that there is an overall decrease in the amount of Red and Orange complex bacteria, but an initial increase in the Green Complex which subsequently decreases from the 3 to 6 month mark leading to a final overall decrease. The control group tended have a better response to AA, TF, CR, and CS while the test group seemed to have a greater reduction in the amount of PG, TD, EN, FN, PI, PM and EC.



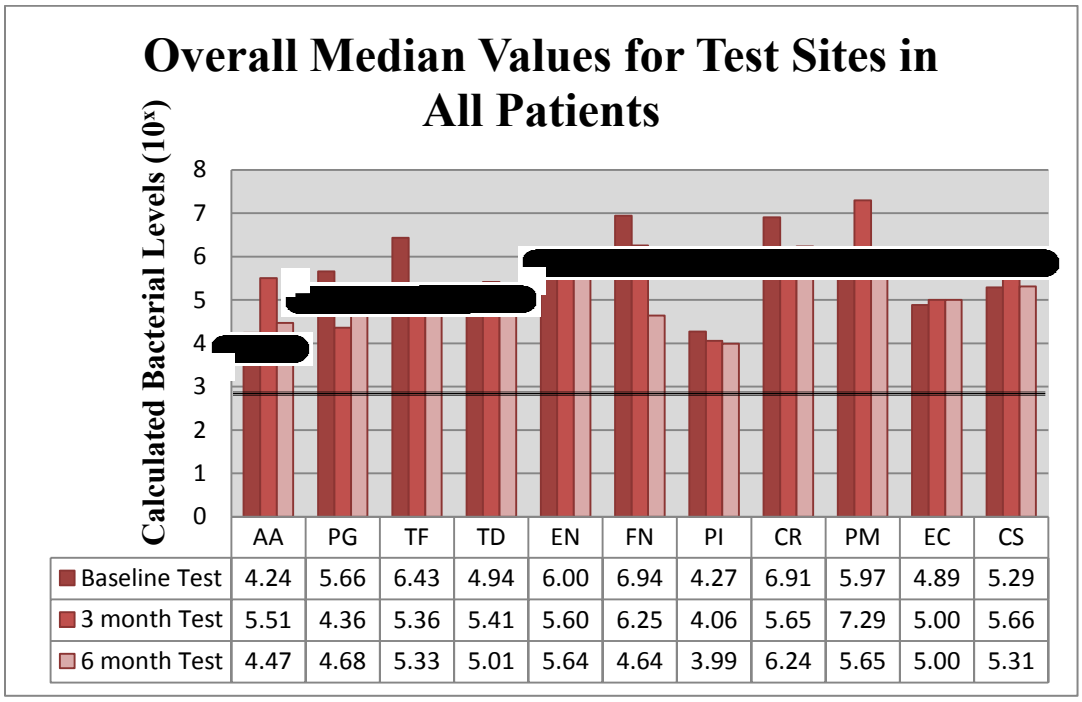
	AA	PG	TF	TD	EN	FN	PI	CR	PM	EC	CS
x Baseline Control	3.88	6.30	6.36	5.13	6.99	5.90	4.18	7.60	5.76	4.99	5.32
x Baseline Test	4.24	5.66	6.43	4.94	6.00	6.94	4.27	6.91	5.97	4.89	5.29

Figure 7: Overall Median Baseline Values from PCR Analysis: Control vs. Test



	AA	PG	TF	TD	EN	FN	PI	CR	PM	EC	CS
Baseline Control	3.88	6.30	6.36	5.13	6.99	5.90	4.18	7.60	5.76	4.99	5.32
3 month Control	4.35	5.40	5.55	5.58	6.22	4.90	4.06	6.56	7.14	5.37	5.56
6 month Control	3.30	6.07	5.73	6.12	6.63	4.93	4.09	6.09	5.71	4.99	5.81

Figure 8: Overall Median Values from PCR samples for Control Sites in All Patients



	AA	PG	TF	TD	EN	FN	PI	CR	PM	EC	CS
Baseline Test	4.24	5.66	6.43	4.94	6.00	6.94	4.27	6.91	5.97	4.89	5.29
3 month Test	5.51	4.36	5.36	5.41	5.60	6.25	4.06	5.65	7.29	5.00	5.66
6 month Test	4.47	4.68	5.33	5.01	5.64	4.64	3.99	6.24	5.65	5.00	5.31

Figure 9: Overall Median Values from PCR analysis for Test Sites in All Patients

II.4.2.1 The Red Complex

The Red Complex consists of *Porphyromonas gingivalis* (PG), *Tannerella forsythia* (TF), *Treponema denticola* (TD). Addressing the control and test sites overall, both groups have high levels of PG, TF, and TD at baseline (Figure 7). An appreciable decrease of PG and TF can be seen from baseline levels to the 3 month sample in both groups (Figure 8, 9). However, in Figure 7, although the control group had a decrease in PG and TF at 3 months, the levels are still considered in the high bacterial load category. Interestingly, in the control group, TD actually increased throughout the study. In the test group from Figure 9, both PG and TF decreased, with PG entering the low detection limit in the 3 and 6 month mark. TF decreased from baseline and maintained a comparable

drop at 6 months, but the overall level is still considered high. Another interesting point is TD increasing from a low detection level at baseline (4.94) to a high detection limit at 3 month, only to drop again slightly to a near low detection level (5.01).

II.4.2.2 The Orange Complex

The Orange Complex evaluated consisted: *Eubacterium nodatum* (EN), *Fusobacterium nucleatum/ periodonticum* (FN), *Prevotella intermedia* (PI), *Campylobacter rectus* (CR), *Peptostreptococcus micros* (PM). From Figure 8, EN and CR are the only bacteria noted at a high limit at baseline for the control group. EN decreased slightly at 3 months, but rebounded at 6 months all while still considered in a high bacterial load category. CR dropped drastically at 3 months and continued at 6 months, but remains in the high load group overall. FN initially started in the low category, but decreased at 3 months and remained nearly consistent at 6 months. PI remained nearly consistent throughout the study in the low detection category. PM interestingly increased dramatically from baseline to 3 months, but returned to near baseline values at 6 months.

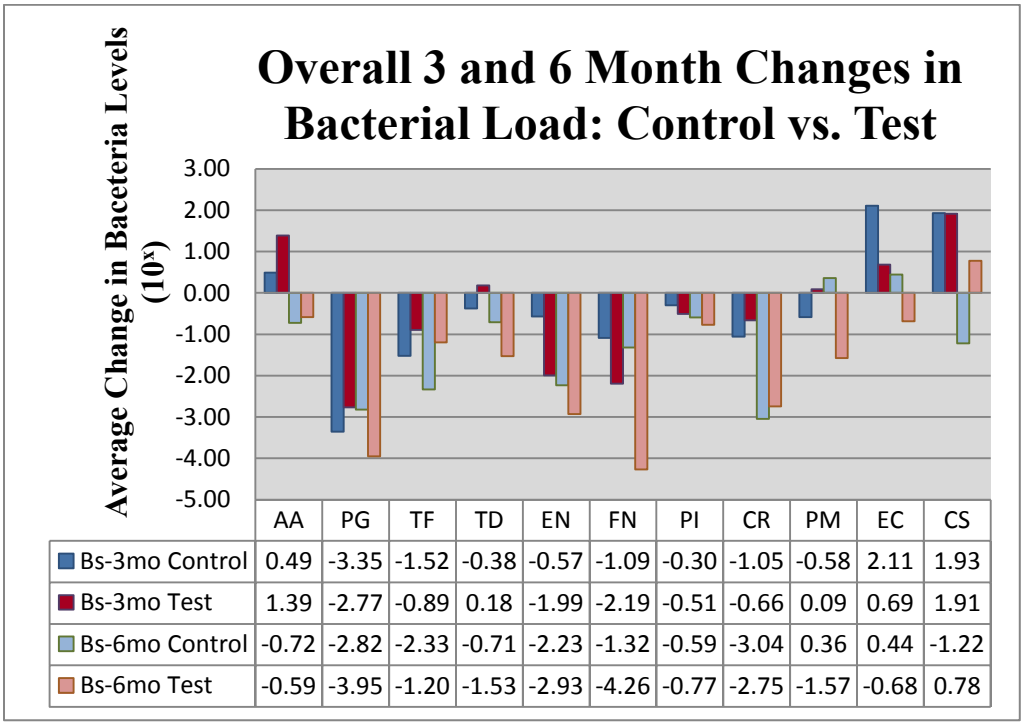
From Figure 9, EN dropped from baseline to 3 months and remained consistent at 6 months for the test sites. FN initially started in a high category (6.94) drops at 3 months and continued to decrease even further, entering the low detection category at 6 months. PI remained nearly consistent throughout the study. CR decreased initially, but rebounded to high detection levels at 6 months. PM, consistent with the trend noted in the control sites, increased drastically from baseline to 3 months, but dropped again to below baseline levels at 6 months.

II.4.2.3 The Green Complex

Aggregatibacter actinomycetemcomitans (AA), *Eikenella corrodens* (EC), *Capnocytophaga* species (*gingivalis, ochracea, sputigena*) (CS) were evaluated for the Green Complex. As seen in Figure 8, the control group had AA increase from low to a high level at 3 months, but dropped below baseline levels at 6 months. EC trended similarly while CS consistently increased throughout the study, but both EC and CS remained within the confines of the low detection limit. From Figure 9, the test group responded like the control group, but at higher spike in AA is seen from baseline to 3 months that remained higher than baseline levels at 6 months.

II.4.2.4 Overall Analysis between Groups

When analyzing between test and controls, Figure 10 depicts changes from Baseline to 3 month and Baseline to 6 month for both groups. The trends noted suggest that there is an overall decrease in the amount of Red and Orange complex bacteria, but an initial increase in the Green Complex which subsequently decreases from the 3 to 6 month mark leading to a final overall decrease. The control tended have a better response to AA, TF, CR, and CS while LANST seemed to have a greater reduction in the amount of PG, TD, EN, FN, PI, PM and EC**.** For this case series, LANST performed better in reducing PG, EN, FN, PM, EC while S/RP had better results in reducing TF and CS; however, no statistical significance was found.



	AA	PG	TF	TD	EN	FN	PI	CR	PM	EC	CS
Bs-3mo Control	0.49	-3.35	-1.52	-0.38	-0.57	-1.09	-0.30	-1.05	-0.58	2.11	1.93
Bs-3mo Test	1.39	-2.77	-0.89	0.18	-1.99	-2.19	-0.51	-0.66	0.09	0.69	1.91
Bs-6mo Control	-0.72	-2.82	-2.33	-0.71	-2.23	-1.32	-0.59	-3.04	0.36	0.44	-1.22
Bs-6mo Test	-0.59	-3.95	-1.20	-1.53	-2.93	-4.26	-0.77	-2.75	-1.57	-0.68	0.78

Figure 10: Overall 3 and 6 Month Changes in Bacterial Load: Control vs. Test

II.5 Discussion

In this case series, a novel approach was utilized in an attempt to apply the adjunctive use of a CO_2 laser intrasulcularly in a non-surgical manner for the treatment of moderate to severe, chronic periodontitis. Within the confines of this study, the adjunctive use of CO_2 laser decontamination was not clinically nor statistically significantly better than scaling and root planing alone for the treatment of moderate to severe, chronic periodontitis in selected teeth when assessing clinical parameters. However, LANST had a propensity to reduce PG, EN, FN, PM, EC bacterial levels more than S/RP alone over a six month period when analyzed by multiplex PCR analysis.

The non-surgical results from this case series are in agreement with other non-surgical studies. After non-surgical therapy, Morrison *et al* reported a 0.96mm pocket depth reduction in sites with initial probing depths of 4-6mm [101]. Kaldahl *et al* reported sites with initial probing depths from 5.0-6.0mm had a 1.23mm reduction in probing depths with 0.96mm gain in clinical attachment 3 months afterwards [128]. Pope *et al* saw a probing depth reduction of 1.8 mm (1 mm gain in clinical attachment) overall in their study using a CO_2 laser for de-epithelialization in combination with S/RP, but with an increase in recession [187]. In the current case series, when the authors combined the test and control sites, there was a 1.51 $\pm$0.003 mm overall reduction in probing (1.28 $\pm$0.004 mm clinical attachment gain) for sites with initial probing depths $\geq$ 4 mm from baseline to 6 months (Table 9). One interesting thing to note is that in sites with probing depths $\geq$ 4 mm at baseline, the laser group had a slightly better gain of CAL of 1.46 $\pm$ 0.105 mm compared the control sites at 1.09 $\pm$ 0.104 mm. This average difference of nearly 0.4 mm may be a considered a moderate benefit for adjunctive therapy when utilizing the criteria from the ADA's Council of Scientific Affairs (Table 9) [188]. However, this needs to be verified with larger sample sizes with better plaque control between visits.

Many recent reports of Nd:YAG, Er:YAG, and Er,Cr:YSGG laser involve inserting a laser tip into the sulcus so that the laser irradiates the sulcular epithelium and root surface. To the author's knowledge, this is the first published report of using a conditioned, ablative CO_2 laser beam placed directly into the sulcus. However, the results of this case series are in agreement with studies that report positive gains from laser therapy inside the sulcus, but not statistically significantly superior to S/RP alone.

Several factors may account for this. Although Breininger *et al* states that a single session of S/RP can yield a significant reduction in bacterial populations even without complete removal of all sub-gingival calculus, it is possible that plaque control was a factor in the outcome of this study [88]. Plaque index was recorded and by having the patients return every 10 days for plaque control and prophylaxis for the first month after initial therapy, a general trend was noted for a better result for the side receiving the LANST protocol. However, an observation from the 3 to 6 month time frame was an increase in detrimental clinical parameters in patients with a lack of ideal plaque control. With the noted increase in plaque scores seen for all the patients, this lack of oral hygiene can be a critical deterrent of healing with neither group reaching the ideal 85% plaque free percentage at any time point. This can be supported by the literature showing the either surgical and non-surgical therapies are effective in eliminating gingivitis and reducing probing depths if the subgingival plaque is eliminated and re-infection is deterred [189].

Another factor is the relatively small sample size (n=14). However, the report of PCR analysis of 9 patients for known periodontal pathogens is advantageous in seeing any possible changes to the periodontal environment throughout the study. However, several other drawbacks must be considered. Although the PCR analysis will detect bacterial RNA within the sulcus, there is no way to differentiate between live, thriving bacteria or just bacterial remnants present within the sulcus. Considering the split mouth design, there is the possibility of cross over contamination from sites that received only S/RP which could "re-infect" LANST sites. The recurrence of several periodontal

pathogens after 3 months appears to be in consensus with the literature. Magnusson reported that in the absence of oral hygiene, spirochetes and motile rods were reestablished in 4 to 8 weeks [107]. Mousques observed that after a single session of S/RP, without proper oral hygiene, there was a return to baseline values by 3 months [110]. In a study of 12 patients with moderate probing depths (4-6 mm), Tabita *et al* noted the development of subgingival plaque within 14 days, even with daily professional care [112]. A future design could be a case controlled study that allows for matched subjects to undergo either S/RP or LANST. It must also be noted although several bacteria in the study appeared to decrease over the time; several (TF and AA) were very resilient and maintained high values at multiple time points for both groups. PM even increased from baseline to 3 months, just to return to near baseline levels at 6 months. There is a trend for nearly all bacteria species except AA, TD, PM, EC and CS to decrease after LANST after 3 months. In this case series, the LANST protocol was only performed at baseline. It would be interesting to see if these downward trends would continue if LANST was performed at a 3 month periodontal maintenance appointment.

Another observation noted during the study was that patients tended to report less sensitivity on the side that had received the LANST protocol, but no attempt was made to officially survey the patients' subjective responses to therapy. In addressing the subjective decrease in sensitivity by the patient, future research would include a visual analog scale, but also in regards to evaluate possible surface changes to the root surface. From the literature, Pogrel *et al* found that when using a Xanar Articulator CO_2 laser with a 1 mm focused lens at 17.5 W (2320 W/cm^2), the tissue necrosis lateral to the incision

line was dependent on the water content of the tissue. They report a mean width of necrosis lateral to the incision was 85.9 μm for epithelium, 51.1 μm for loose connective tissue, and 96.1 μm for dense connective tissue [172]. Although the LANST protocol uses a conditioned ablative beam, it is uncertain if the beam may cause any changes on the root surface. In a study by Almehdi *et al*, the authors found that direct irradiation of a root surface at 1.0 W without coolant in a non-contact focused mode for 2 seconds, the histological and scanning electron micrographs of the surface revealed numerous microcracks along with melted structures [190].

One possible explanation may be answered by Barone *et al.* The authors subjected extracted root surfaces to different modes of CO_2 beams in an *in vitro*, scanning electron microscope study. When comparing an 8 W, continuous mode with a focused beam of 0.8 mm to a 2 W, pulsed mode at 4 Hertz, non-focused beam of 4 mm aligned directly to the root, the defocused mode did not result in the same amount of damage to the root surface. While the continuous mode created craters and fissures, the defocused beam created smooth, flat surface that sealed the dentinal tubules [184]. To the author's knowledge, there are no studies that look at the effect of a continuous, 8 W CO_2 ablative beam lateral to a root surface. Ideally the tip is kept parallel to the root surface, but the heat may be decontaminating and sealing dentinal tubules, this may result in a desensitization of the root surface.

Further research is needed to evaluate the efficacy of CO_2 laser therapy as an adjunct to non-surgical therapy. For future studies, the authors recommend use in patients with either established or adequate plaque control during maintenance

appointments in residual probing depths or sites with consistent BOP. It is also recommended to include the use of a visual analog scale to account for subjective responses in regards to sensitivity or discomfort during or after therapy. A full mouth debridement with the laser appears to be favorable to a split mouth design.

III. CONCLUSION

Within the confines of this six month study, sites treated with the LANST procedure tended to show a greater decrease in probing depths and greater gains in clinical attachment levels; however, the results were not statistically significantly better than scaling and root planing alone. The decrease in several suspected periodontal pathogens for the first 3 and 6 months after therapy appears very promising. However, further research is needed to evaluate the efficacy of the LANST protocol in larger, clinical studies.

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