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Perio Protect™ Treatment of Chronic Periodontitis Reduces Biofilm and Inflammation

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ABSTRACT

Periodontal health exists when a small numbers of predominantly Gram-positive aerobes, with fewer Gram-positive facilitative and a small number of obligate anaerobes live supragingival and subgingival in sulci less than 3 mm. The biofilm and host cohabitate in a commensal or mutualistic relationship without a host inflammatory response.

Periodontal disease occurs when there is a shift in critical wound composition of the periodontal subgingival microbiome associated with host micro-environment changes. The oxygen saturation decreases, and an imbalance (dysbiosis) occurs where the more virulent anaerobic pathogenic microorganisms predominate at the expense of aerobic commensal microorganisms, fostering a chronic wound, resulting in an inflammatory immune response.

Existing treatments focus on controlling plaque but fail to manage the immune inflammatory reaction. The Perio Protect Method™ manages plaque and inflammation through subgingival delivery of 1.7% hydrogen peroxide (Perio Gel™) and antioxidants. Perio Trays™ are FDA cleared medical devices manufactured with customized seals and extensions which compress the wound and overcome crevicular flow and deliver medications better than other dental appliances to the source of the dental infection. Perio Gel™ generates 5.3 X oxygen saturation in the Perio Tray™ comparable to the oxygen saturation in a hyperbaric chamber. Thr Perio Protect Method™ manages obligate anaerobes, accelerates tissue recovery, healing, and repair, establishes a microenvironment reversing dysbiosis, and improves systemic LpPLA2 and hs-CRP levels. The numbers of virulent facultative and obligate anaerobes decrease and are replaced with fewer aerobic bacteria found in healthy tissues. Anti-inflammatory medications neutralize the host inflammatory response, decrease tissue damages, and augment osteogenic improvements.

Managing periodontal inflammation is medically important because emerging evidence demonstrates a strong relationship between periodontal inflammation and numerous systemic conditions including, but not limited to, atherosclerosis, stroke, Alzheimer's disease, type 2 diabetes, obesity, and rheumatoid arthritis. A common link in these systemic conditions with periodontitis is an increased systemic inflammatory burden, which exacerbates other co-existing inflammatory conditions leading to related diseases.

Treatments convert the microenvironment from disease to health (reverse dysbiosis) and address both the etiology (biofilm) and the inflammatory host responses. The Perio Protect Method™ is a treatment regimen that becomes a long-term periodontal homecare system.

Keywords
Critical wound composition**,** Reverse dysbiosis, Anti-inflammatory, Osteogenic management, Perio Protect Method, Biofilm management, Systemic inflammatory burden, Lp-PLA2, hs-CRP.

Changing Periodontal Treatment Philosophies

A healthy biofilm in periodontal pocket of 3 mm or less has a normative oxygen tension with a low number of predominant Gram-positive aerobic biofilm with few facultative anaerobes and some obligate anaerobic bacteria without a significant host inflammatory response [1]. Periodontal disease is defined as an increase in the number of subgingival microorganisms and a shift (dysbiosis) from a healthy biofilm to an increased number of predominantly Gram-negative facultative and obligate anaerobes, which cause a host inflammatory response [2].

A possible explanation of the shift in the biofilm from supporting health to disease relates to microenvironmental changes, which occur in the periodontal pockets. A correlation exists between the number and type of subgingival microbes, microenvironmental changes, and the oxygen tension in chronic wounds. An increasing number of aerobic and facultative anaerobes use up oxygen, and oxygen tensions decreases. Anaerobes increase as the oxygen tension decreases. The presence of anaerobes is important to chronic wound (periodontal) therapy as anaerobes regrow in a matter of days, increase in virulence over time causing a chronic wound state, which continues or accelerates the infectious processes.

Critical wound colonization in chronic wounds refers to the quantity and type of infectious agents as well as the host responses. An increased critical wound colonization of more virulent bacteria relates to increased host inflammatory responses. The biofilm pathogens create unique immunologic challenges to which they are already adapted, such as reducing the oxygen concentration in a periodontal pocket. The decreased oxygen tension favours the facultative and obligate anaerobes while limiting the aerobic bacteria [3].

The shift from health to disease involves a change from a low number of Gram-positive aerobic and facultative anaerobic bacteria (10^2 -10^3), which is replaced by a greater number (10^4 – 10^5) of Gram-negative anaerobic microorganisms (dysbiosis). The chronic wound periodontal pocket oxygen tension decreases as dysbiosis occurs [4], where a greater number of Gram-negative anaerobic bacteria cause local and systemic chemokines and cytokines responses, and neutrophils and other immune cells decrease their antimicrobial function [5].

In chronic conditions, the chemokines and cytokines have adverse effects as compared to acute circumstances. Gram-positive and Gram-negative bacteria equally induce IL-1 beta, but Gram-positive bacteria generate twice as much TNF-alpha. IL-1 beta is a key mediator of inflammation, which is part of the immune response to pathogens, but excessive IL-1 beta also exacerbates acute tissue injury. TNF-alpha activates cellular responses including cell survival, differentiation, and proliferation, but excessive activation leads to pathologic complications. Gram-negative bacteria induce at least twice as much IL-6 and IL-8. IL-6 contributes to host acute phase immune reactions, but in excess has a pathologic effect on chronic inflammation and autoimmunity [6]. IL-8 is important in neutrophil activation in acute infections, but IL-8 is also important in establishing and preserving the inflammatory microenvironment of the vascular wall in atherosclerosis and within the periodontal tissues in chronic periodontitis [7].

In chronic wound conditions, Gram-negative bacteria produce lipopolysaccharides (LPS) that in combination with IL-8 impair neutrophil phagocytic action [8]. When lipopolysaccharides are introduced systemically, they induce inflammatory cell infiltrate into blood vessel walls, causing vascular smooth muscle proliferation, vascular fatty degeneration, and intravascular coagulations. LPS up-regulates endothelial cell adhesion molecule expression and increases the secretion of interleukin-1 (IL-1), Tumor necrosis factor alpha (TNF-alpha) and thromboxane, which increases platelet aggregation and adhesion, causing the formation of lipid laden foam cells and deposits of cholesterol and cholesterol esters, which are found systemically in patients with atherosclerosis [9].

Gram-negative bacteria pose more host inflammatory complications due to the cell wall of gram-negative bacteria. This increases the risk of toxicity to the host, but this membrane is absent in gram-positive bacteria. Gram-negative bacteria possess porin channels, which can prevent the entry of harmful chemicals and antibiotics like penicillin. These channels can also expel antibiotics out of the bacteria, making it more difficult to treat Gram-negative bacteria. The risk of resistance against antibiotics is higher in Gram-negative bacteria due to the presence of external covering around the cell wall, and Gram-negative bacteria possess both exotoxins and endotoxins while Gram-positive bacteria produce predominantly exotoxins [10].

The contemporary treatment of periodontitis involves a mechanical removal and anti-infective protocols to manage bacterial plaque. Scaling, root planing and mechanical debridement are “gold standards” of existing professional care for plaque management, which is continued by the patient with a homecare regimen of brushing, flossing, oral rinses, and other adjunctive means. Mechanical treatments decrease subgingival biofilm numbers, but treatments “stagnate” with accompanying deterioration in the biofilm profile [11].

Around 6 weeks after scaling and root planing the regrowing biofilm becomes more virulent as pathogen-related components expand as non-pathogen components contract [12]. To counter this increased pathogenicity, conventional anti-infective regimens focus on antibiotics as a treatment adjunct. The use of antibiotic is associated with an increase in adverse patient responses and

an increase in the biofilm antibiotic resistance through multiple antibiotic resistant means [13]. Dentistry, and in particular periodontal treatments are slow to fully adopt post-antibiotic era developments and as a result antibiotic usage remains.

Antibiotic effectiveness in the management of periodontitis is questioned because many antibiotics lack the ability to penetrate the biofilm matrix, and the resistance to antibiotics increases from the remaining bacteria [14]. Mechanically disturbing the biofilm is sometimes used to help antibiotic penetration. Accessing the bottom of the periodontal pocket and maintaining a clinically significant subgingival antibiotic dose for a sufficient time is problematic due to an increased periodontal pocket crevicular flow that removes the antibiotic before it can be effective in microbial management [15].

The host inflammatory responses to the biofilm challenge are responsible for some of the periodontal pathology. Inflammation is central in the change from a healthy condition to a disease state. The inflammation increases as the biofilm undergoes a change from commensal bacteria to a more pathogenic biofilm [16], and the biofilm becomes more virulent following scaling and root planning. The host increasing inflammation to the changed biofilm microenvironment may affect the systemic inflammatory burden [17]. If controlling inflammation is a key in managing periodontal infections [18], then treatment paradigms for chronic wounds like periodontal disease must manage both the biofilm etiology and the host inflammation.

Chronic wound treatments are not the same as acute wound therapy. Removing a splinter is acute wound treatment as the cause of infection is eliminated and the host heals. Mechanical removal of the supra and subgingival biofilm in the periodontal chronic wound decreases the bacterial population, but the biofilm regrows as a more pathogenic community. These results are contra to the American Medical Association (Wound Healing Society) Guidelines for Chronic Wound Management [19]. The "gold standard" of chronic wound management involves controlling the cause of pathology or infection, arresting the wound progression, preventing wound reoccurrence, and promoting regeneration and healing.

The Perio Protect Method™ fulfills the AMA Chronic Wound Guidelines as applied to periodontal treatments. Chronic wound control may involve managing oxygen tension levels that vary from health to disease. When the oxygen level decreases, the oxygen-hemoglobin disassociates providing oxygen to the tissues. If the oxygen level gets too low the tissues die. Not only is the oxygen tension level important, but for oxygen to be delivered to the tissues the distance between the capillaries and the cells is paramount. When the distance between the cells and capillaries increases new capillaries are formed, resulting in an inflammatory state [20].

Oxygen is delivered and maintained in the ischemic periodontal pocket using an FDA cleared medical device (Perio Tray™). The Perio Tray™ delivers and maintains medications deeper into a periodontal pocket than other trays. The custom formed seals direct medications subgingival and correspond to the magnitude of the host injury. The custom formed extensions serve as a wound compression appliance to reduce swelling by returning excess interstitial fluids to the vascular or lymphatic systems [21]. Oxygen tension levels are restored and maintained at greater than normative levels during treatment to overcome hypoxia conditions enabling a shift (reverse dysbiosis) in the predominant periodontal species from a larger number of more virulent anaerobes to a lesser number of more commensal aerobic bacteria [22]. The increased oxygen level comes from the oxidative-reduction reaction of hydrogen peroxide to water and oxygen.

Subgingival 1.7% hydrogen peroxide (Perio Gel™) is antimicrobial by its oxidative/reduction processes forming water and oxygen. In the periodontal pocket the makeup of Perio Gel™ supports an eighteen (18) minute consistent (linear) conversion to oxygen and water. The increased oxygen tension results in micro-environmental alterations from an oxygen depleted (anaerobic) to a 5.3 X oxygen saturation [23] (hyperbaric) environment.

Hyperbaric oxygen therapy increases oxygen concentration to overcome tissue ischemia, decreases surrounding edema and increases neovascularity in ischemic tissues. Hyperbaric oxygen is bacteriostatic and bactericidal at higher concentrations and has been found to neutralize Gram-negative anaerobe exotoxins and promote neutrophil-mediated bacterial killing in hypoxic tissues [24]. Hyperbaric oxygen prevents the release of proteases and free radicals, thereby decreasing vasoconstriction, edema, and cellular damage [25]. Hyperbaric oxygen promotes tissue recovery, improves tissue attributes, decreases periodontal pocket probing depth and bleeding upon probing and decreases tissue inflammation [26].

Oxygen under pressure suppresses circulating macrophages in acute phase, helps increase the number of proliferating and differentiating satellite cells, and promotes tissue regeneration [27]. The conversion of hydrogen peroxide to oxygen contributes to energy demands necessary for the healing, reduces the incidence of infections, acts on signaling pathways that modulate the synthesis of inflammation mediators, antioxidants and growth factors, which empower the recovery processes as inflammation decreases [28].

Anti-inflammatory agents (doxycycline or other anti-inflammatory medications) in a subclinical dose is delivered via the Perio Tray™ and maintained for a sufficient time to decrease periodontal pockets and inflammation in chronic periodontal patients, improve scar tissue formation, collagen formation (increased tensile strength) and angiogenesis [29]. Doxycycline possess systemic effects as it is anti-inflammatory, antioxidant, anti-apoptotic neuroprotective, immunomodulatory, anti-collagenolytic, antiproliferative and inhibits the viability and proliferation of human mast cells [30].

Monocytes and macrophages responding to dysbiosis accelerate the production of cytokines, which increases periodontal inflammation [31]. The secretion of pro-inflammatory cytokines (IL-1 beta, IL-8, and TNF-alpha) by macrophages is decreased significantly with doxycycline, which decreases the number of immuno-positive cells thereby reducing cytokines and hs-CRP, IL-6, and MMP-9 levels [32]. Doxycycline decreases MMP-8 or collagenase-2 in the periodontal pocket. MMP-8 is a potent proteinase implicated in periodontal disease and atherosclerotic plaque. Systemic MMP-8 destabilization facilitates cardiovascular rupture through its proteolytic ability to thin the protecting collagenous fibrous cap lining coronary and other arteries. Doxycycline decreases periodontal MMP-8 and systemic MMP-8 levels [33].

Lipoprotein-associated phospholipase A2 (Lp-PLA2) is a systemic inflammatory marker that indicates patients who may develop atherosclerosis, have a myocardial infarction, stroke, or develop cardiovascular disease. Lp-PLA2 levels decrease in patients treated with the Perio Protect Method™ [34]. Similarly, the systemic inflammatory marker high-sensitivity C-reactive protein (hs-CRP) decreases in patients treated with the Perio Protect Method™ [35]. These studies demonstrate the Perio Protect Method™ lowers some systemic inflammation (Lp-PLA2 and hs-CRP) but additional studies are needed to assess the effects on the systemic inflammatory burden.

Doxycycline Effects on Bone Osteoclasts, Osteoblasts and Bone Regeneration

Doxycycline inhibits osteoclast function, stimulate osteoblastic bone formation, and regulate angiogenesis [36]. Topical doxycycline through intercellular and extracellular activity significantly decreases osteoclastic activity associated with decreased osteoclastic bone resorption, thus diminishing bone loss [37]. Not only is the action of existing osteoclasts inhibited [38] but mature osteoclastic apoptosis is induced, and the formation of new osteoclasts is completely abrogated through doxycycline action on osteoclastic precursor cells [39], further decreasing in the cells responsible for bone loss. Doxycycline increases the proliferation of human bone marrow osteoblastic cells resulting in a significant increase in the number of active osteoblastic cells, yielding an increase in new bone [40].

Directly applied doxycycline modifies bone loss around natural teeth [41] and implants [42] by modifying osteoclastic activity, osteoclastic precursor cell action, and osteoblastic activation. The following illustrates the possible results:

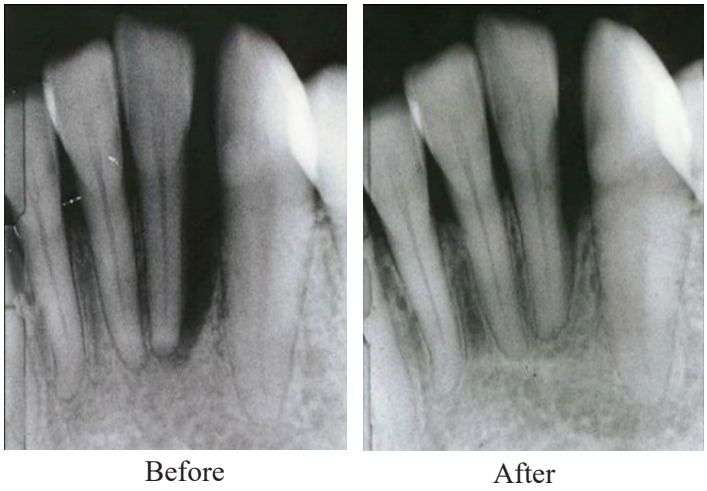

Figure 1: Before and after 19 months of local delivery of doxycycline fostering bone regeneration. D Keller by permission.

Figure 2: Gordon Wilson by permission. Osteogenic sequence around a maxillary lateral incisor using the Perio Protect Method™ to locally deliver doxycycline to an osseous defect with resultant bone regeneration.

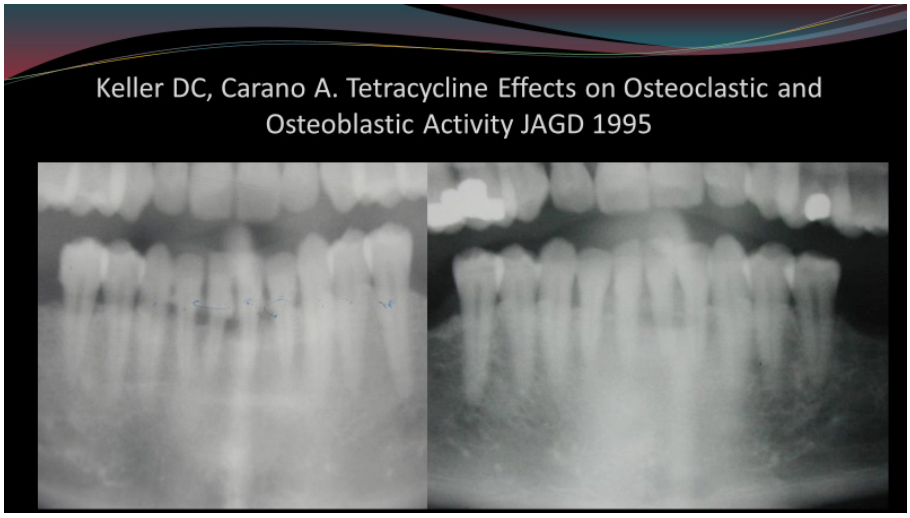

Figure 3: 20+ years of osteogenic management with direct medication delivery doxycycline with the Perio Protect Method™ by permission Dr D Keller

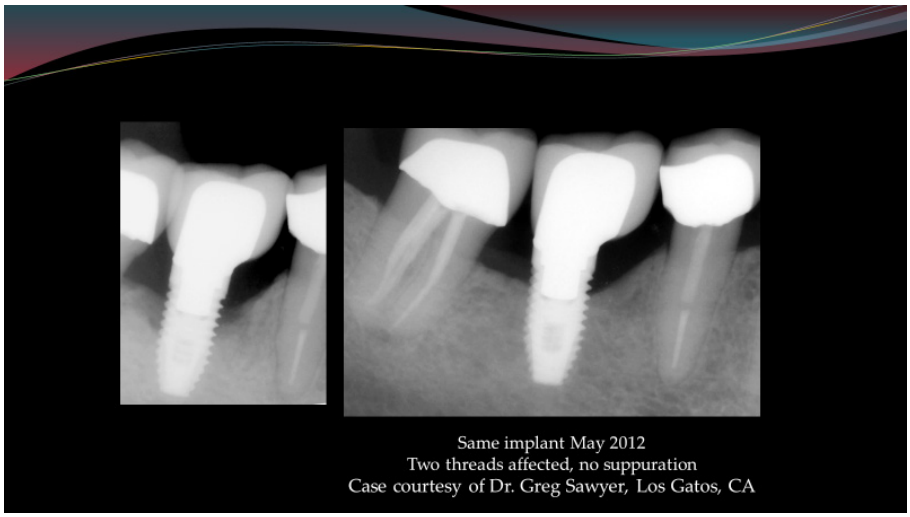

Figure 4: Bone management around implant Dr. Greg Sawyer by permission.

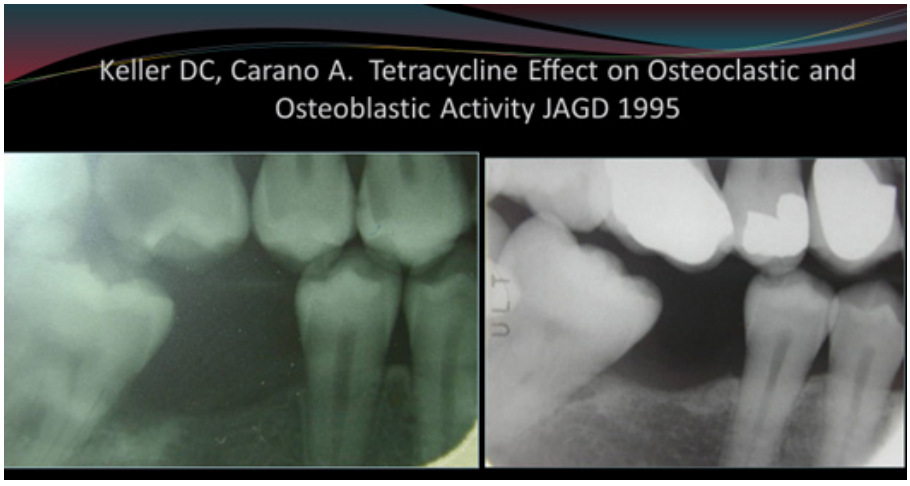

Figure 5: Bone regeneration over 20+ years with the Perio Protect Method™. D Keller by permission

The images of osteogenic management represent conditions where existing osteoclastic activity is decreased by doxycycline introduction into the periodontal pocket with direct medication delivery with the Perio Tray™. Doxycycline treated groups exhibited new bone and connective tissue formation, re-epithelization, and the absence of inflammatory infiltrate resulting in complete re-epithelization and tissue remodeling. This occurs through anti-inflammatory and cytokine inhibitory properties, increasing osteoblastic activity, decreasing osteoclastic activity, inactivating the formation of new osteoclasts, activating immuno-positive cell responses resulting in bone repair in periodontal diseases [43], which is substantiated in similar studies, including around implants [44-46].

Periodontitis may affect systemic inflammatory burdens. An inflamed junctional epithelium in a periodontally involved mouth with 28 teeth and widespread 5 mm pockets includes a surface area between 20 cm^2 to 75 cm^2 [47]. These chronic wounds expose the underlying capillary system to inflammatory toxins, chemokines, cytokines, and microbiologic invasion. These inflammatory products are elevated in the blood stream following mastication on infected gum tissues [48].

Class A evidence supports a correlation between periodontal high-risk pathogens and the pathogenesis of atherosclerosis [49]. Oral perio-pathogens have been isolated from heart valves in patient's undergoing valve replacement surgery [50] demonstrating a possible portal of entry via inflamed periodontal capillaries to toxins and bacterial invasion. Systemic inflammation is elevated by periodontal procedures, which increase chemokines, cytokines, toxins, and bacterial entry into the blood stream [51].

The presence of chronic inflammation (inflammatory priming) is a cumulative process of inflammatory damage that increases over time and contributes to the pathogenesis of other inflammatory conditions. The cumulative inflammatory burden increases with more sources of chronic inflammation, contributing to increased risk for developing or exacerbating other inflammatory conditions such as cardiovascular disease, diabetes, rheumatoid arthritis [52,53]. Chronic inflammation and the cumulative inflammatory burden have been shown to be a predictive factor for multiple systemic conditions such as cardio-metabolic, neurodegenerative, autoimmune diseases and cancer [54].

Untreated periodontitis inflammation contributes to the cumulative inflammatory burden of an individual and has the potential to impact other inflammatory conditions. The relationship between periodontitis and other systemic conditions is a "two-way street" where changes in one area may influence the other [55]. The underlying concept of periodontal treatment is that periodontal inflammation, and the periodontal microbiome contribute to the overall systemic burden of inflammation and infection [56,57] affecting the severity and progression of other chronic inflammatory conditions.

The potential exists for management of periodontal inflammation to impact other inflammatory conditions through managing dysregulated inflammation in treating some systemic diseases. Controlling periodontal inflammation may significantly contribute to controlling systemic inflammation and promote overall health. This modifies the focus of periodontal treatment towards reduction of inflammation rather than eradication of infection and may help explain the decrease in systemic lipoprotein-associated phospholipase A2 (Lp-PLA2) and high-sensitivity C-reactive protein (hs-CRP). Controlling periodontal inflammation may decrease other systemic markers. Additional direct delivery medications may optimize both local and systemic disease management, maximizes recovery, and maintain long-term protection. Additional studies are needed to confirm these possibilities.

Conclusion

Conventional periodontal mechanical treatments decrease plaque initially but facilitate the development of a more virulent post-treatment biofilm. The Perio Protect Method™ is a periodontal treatment and long-term homecare maintenance system, which complies with the American Medical Association, Chronic Wound Guidelines. Direct delivery medications maintained in place for a sufficient time control the cause of infection, arrest the wound progression, promote regeneration and healing, and prevent wound reoccurrence. The Perio Protect Method targets the microbial biofilm and the inflammatory response by direct medication delivery to the periodontal pocket to reverse dysbiosis.

The Perio Protect Method™ delivers antimicrobial 1.7% hydrogen peroxide (Perio Gel™) to the periodontal pocket using a custom formed delivery system, FDA cleared medical device (Perio Tray™). The Perio Tray™ delivers and maintains medication in the periodontal pocket better than other dental trays. Hydrogen peroxide penetrates the biofilm matrix and increases the oxygen saturation 5.3 X in a linear concentration for eighteen minutes. This 5.3 X oxygen concentration reverses the hypoxic periodontal pocket to an oxygen-enhanced microenvironment like hyperbaric oxygen. An increased oxygen concentration helps manage the biofilm, helps control inflammation, arrests the wound progression, and promotes healing.

A subclinical dose of doxycycline serving as an antioxidant/anti-inflammatory in combination with an increased oxygen tension helps control inflammation by decreasing a larger number of anaerobes, which are replaced by fewer Gram-positive aerobic and facultative anaerobes. The increased oxygen tension and anti-inflammatory effects decrease chemokines, cytokines, toxins, and the number of immuno-positive cells, thereby reducing hs-CRP and MMP-9 levels. These changes reverse dysbiosis from disease to conditions found in healthy periodontal tissues.

Doxycycline, via osteogenic control, augments new bone formation and stronger connective tissue regeneration around teeth and implants. The Perio Protect Method™ manages periodontal

inflammation and decreases systemic inflammatory markers Lp-PLA2 and hs-CRP. Further research is needed to evaluate additional medications and how managing periodontal inflammation may improve systemic inflammation, oral and systemic health.

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