T.C
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CLINICAL AND MICROBIOLOGICAL EFFECTS OF PROBIOTIC CONTAINING LOZENGES IN COMPARISON TO SUB-ANTIMICROBIAL DOSE DOXYCYCLINE IN THE TREATMENT OF CHRONIC PERIODONTITIS: 3-MONTH FOLLOW-UP

PhD Thesis

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ONAY

Bu tez Yeditepe Üniversitesi Lisansüstü Eğitim-Öğretim ve Sınav Yönetmeliğinin ilgili maddeleri uyarınca yukarıdaki jüri tarafından uygun görülmüş ve Enstitü Yönetim Kurulu'nun 16./12./2015 tarih ve 31-5 sayılı kararı ile onaylanmıştır.

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I. SUMMARY

The aim of this study is to evaluate clinical and microbiological effects of *Lactobacillus reuteri (L.reuteri)* containing lozenges in comparison with the usage of sub-antimicrobial dose-doxycycline (SDD) containing tablets as adjunctive to initial periodontal therapy in chronic periodontitis (CP) patients

A total of 45 patients, with 2 teeth in each quadrant having at least one approximal site with a probing depth (PD) of 5-7 mm and gingival index (GI) of ≥2, were selected and divided randomly into 3 groups. Group I received scaling and root planning (SRP) + *L. reuteri* containing lozenges, whereas Group II received SRP + SDD containing tablets, and Group III received SRP + placebo. Plaque index (PI), GI, PD, relative attachment level (RAL) and bleeding on probing (BoP) were measured for clinical evaluation. Microbiological sampling was performed at baseline and at day 90, and was analyzed by culture method. Total viable count (TVC) and proportions of obligate anaerobic bacteria were evaluated.

The paired sample t test was used for intra-group comparison of the clinical parameters and the proportions of obligate anaerobic bacteria, whereas the Wilcoxon sign test was used for the TVC values. One-way ANOVA test was used for the inter-group comparisons of mean differences of clinical parameters and proportions of the obligate anaerobic bacteria (%), while Kruskal-Wallis Test was used for the TVC values. Statistical significance was set as $p<0.05$. Tukey test was used to evaluate the comparisons of the clinical parameters and proportions of obligate anaerobes in pairs, whereas Mann–Whitney U-test was used for the evaluation of TVC values in pairs. Statistical significance was set as $p<0.05$.

All treatments resulted in statistical significantly improvements in terms of the clinical and microbiological parameters at the end of the observation period.

Intergroup comparisons of the mean differences of PI, GI, BoP, PD and attachment gain revealed statistical significance in favor of both SRP + Probiotic and SRP + SDD groups ($p<0.05$) at the end of day 90. Intergroup comparisons of TVC values revealed significance in favor of the SRP + Probiotic group at the end of day 90 ($p<0.05$). Intergroup comparisons of proportions of obligate anaerobic bacteria revealed statistical significance in favor of the SRP + Probiotic and SRP + SDD groups when compared to the SRP + Placebo between days 0 and 90 ($p<0.05$).

Within the limitation of this study, it can be concluded that *L. reuteri* containing lozenges and SDD containing tablets might be adjunctive useful agents for the improvement of periodontal health in CP patients.

Key words: SRP, Chronic Periodontitis, Probiotics, Sub-Antimicrobial Dose-Doxycycline, Host Modulation.

II. DECLARATION

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which has been accepted for the award of any other degree except where due acknowledgment has been made in the text.

Sarah ALSULAIMANI BÜYÜKDAĞ

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IV. CONTENTS

V.ABBREVIATION

A.a: *Aggregatibacter actinomycetemcomitans*

ADA: American Dental Association

BMP: Bone Morphogenetic Proteins

BoP: Bleeding on Probing

CAL: Clinical Attachment Loss

CFU: Colony Forming Unit

CP: Chronic Periodontitis

EMP: Enamel Matrix Proteins

ELISA: Enzyme Linked Immunosorbent Assay

FDA: Food and Drug Administiration

GBI: Gingival Bleeding Index

GF: Growth Factors

GI: Gingival Index

HMT: Host Modulation Therapy

IBS: Irritable Bowel Syndrome

IL-: Interleukins

IL-1ra: Interleukins Receptor Antagonist

L.reuteri: *Lactobacillus reuteri*

MMPs: *Matrix Metalloproteinases*

MRONJ: Medicine-Related Osteonecrosis of the Jaw

NG: Neosteline Green

NSAIDs: Non-Steroid Anti-Inflammatory Drugs

OHI: Oral Hygine Instruction

PD: Probing Depth

P.g: *Porphyromonas gingivalis*

PGE_2: Prostaglandin E_2

PI: Plaque Index

P.i: *Provetella intermedia*

RT-PCR: Reverse Transcription Polymerase Chain Reaction

SDD: Sub-Antimicrobial Dose-Doxycycline

SRP: Scaling and Root Planning

TCs: Tetracyclines

T.d: *Treponema denticola*

T.f: *Tannerella forsythia*

TIMPs: Tissue Inhibitor of Metalloproteinases

TNF-α: Tumor Necrosis Factor Alpha

TVC: Total Viable Count

WHO: World Health Organization

VI. FIGURES and TABLES LIST

FIGURES

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1. INTRODUCTION AND AIM

The human mouth harbors millions of highly diverse microbial organisms. These include approximately 700 species of bacteria, as well as viruses, fungi and protozoa (1), and there is a natural balance between these organisms and the host immune system. The combination of many reasons, such as the increase in the proportion of pathogenic bacteria, and decrease in the beneficial bacteria in addition the presence of susceptible individuals leads to disrupt the balance and eventually leads to periodontitis (2).

In the oral cavity, bacteria grow in complex polymicrobial associations known as biofilms. Darveau et al. (3) has firmly established that the dental plaque should be thought of as a biofilm, and that periodontitis should be considered a biofilm-associated disease (4). Due to this knowledge, factors that facilitate plaque accumulation retain microorganism in proximity to the periodontal tissues, providing an ecological niche for biofilm formation, thereby these factors should be eliminated (5). Conventional periodontal treatment, aims to disrupt the biofilm, reduce the number of periodonto-pathogens, and remove all deposits from root surfaces (6), which prevents further damages and re-establish the tissue to a more stable level (7, 8). However, this therapy remains insufficient in the presence of deep periodontal pockets, anatomic grooves and root concavities. Therefore many treatment alternatives have been suggested in order to improve the outcomes of the therapy. Antimicrobial treatment approaches such as antibiotics, antiseptics, laser and photodynamic therapy in addition to mouth rinses and gels has been used as adjunctive to scaling and root planning (SRP) (9-11). However, due to the development of resistance to a range of antibiotics by some important pathogens, are not considered first choice of adjunctive. These developments have encouraged researchers in various field of health care to evolve alternative approaches.

One of the promising approach; is the host modulation therapy (HMT), which, as the name suggest aims to modulate the host by suppressing the inflammatory response. A variety of drugs have been evaluated as host modulatory agents including non-steroid anti-inflammatory drugs (NSAID), enamel matrix proteins (EMP), growth factors (GF), bone morphogenetic proteins (BMP), Bisphosphonates, and sub-antimicrobial dose-doxycycline (SDD) (12). Among them SDD is a 20 mg dose of doxycycline that is indicated as an adjunct to SRP in the treatment of chronic periodontitis (CP). The 20 mg dose exerts its therapeutic effects by enzyme, cytokines and osteoclast inhibition rather than any antibiotic effect. SDD is the only HMT agent

approved by Food and Drug Administration (FDA) and accepted by the American Dental Association (ADA)(13).

On the other hand, another promising agent is probiotics which according to World Health Organization (WHO), are defined as live microorganisms which, when administered in adequate amounts, confers a health benefit on the host (14). The mechanism of action of probiotics is still unclear but it is estimated that their action is related to their ability to modulate the host immune system, their direct effects against pathogenic bacteria and their indirect effects against pathogenic bacteria (15).

In the literature there is no study evaluating the host modulatory effects of probiotics in comparison with SDD in the treatment of CP. Therefore we aimed to evaluate the effects of both agents in comparison to each other in CP patients in a 3-month follow up period on clinical and microbiological parameters.

2. LITERATURE REVIEW

2.1. Periodontal Disease

Periodontal disease could be noted as a multifactorial, polymicrobial infection initiated by the accumulation of specific bacteria present in the dental biofilm especially in, and around the gingival crevice region, leading primarily to gingival inflammation. In case of persistence it may lead to the destruction of periodontal ligament and the alveolar bone in susceptible individuals (16).

Over the years three main hypotheses have been proposed in order to understand the pathogenesis of periodontal disease (17). The first theory was the **non-specific plaque hypothesis**; it proposed that the accumulation of bacteria adjacent to the gingival margin led to gingival inflammation and subsequent periodontal destruction. The idea was that the plaque amount and the toxic products are what determine the development of the disease by overwhelming the host's defenses. While this hypothesis explained the development of gingivitis, it failed to explain the development of periodontitis due to the knowledge that not all gingivitis progress to periodontitis and some individuals with high amounts of accumulated plaque do not show any signs of periodontitis (18). In the 1970s the **specific plaque hypothesis** was introduced, which proposed that subgingival plaques differ in their pathogenic potential, which was dependent upon the presence or an increase of specific pathogenic bacteria and their toxic products within the subgingival plaque. Studies identified clear changes in plaque composition in which the presence of gram-negative, obligate anaerobic species were associated with an increase in periodontal pocket depths (19). These studies culminated with the identification of specific microbial groups within dental plaque (20). Six closely inter-related groups of microbes were reported, with the 'red complex' consisting of *Tannerella forsythia* (*T.f*), *Porphyromonas gingivalis* (*P.g*) and *Treponema denticola* (*T.d*), and these bacteria were significantly associated with the clinical features of periodontitis. However, putative periodontal pathogens (such as *P. g* and *T. f*) are frequently found in healthy periodontal tissues which question's them being true pathogens. The inconvenience of this hypothesis led the researchers to develop an alternative hypothesis to answer the pathogenesis of periodontal disease, and in the early 1990s the **ecological plaque hypothesis** was proposed. In this hypothesis it is proposed that the subgingival environment dictates or selects the specific microbial composition and this, in turn, drives the change from health to disease. Specifically, this hypothesis proposes that the nonspecific accumulation of plaque leads to inflammation

within the gingival tissues and to the development of gingivitis. This leads to environmental changes within the gingival sulcus, which in turn favor the growth of gram-negative and proteolytic species of bacteria. These changes lead to further inflammatory and immune-mediated tissue changes, further environmental changes and tissue destruction, culminating in a predominance of periodontal pathogens and a greater degree of tissue damage. Hence, the inflammation within the tissues drives the microbial changes and not *vice versa*, as is the current dogma.

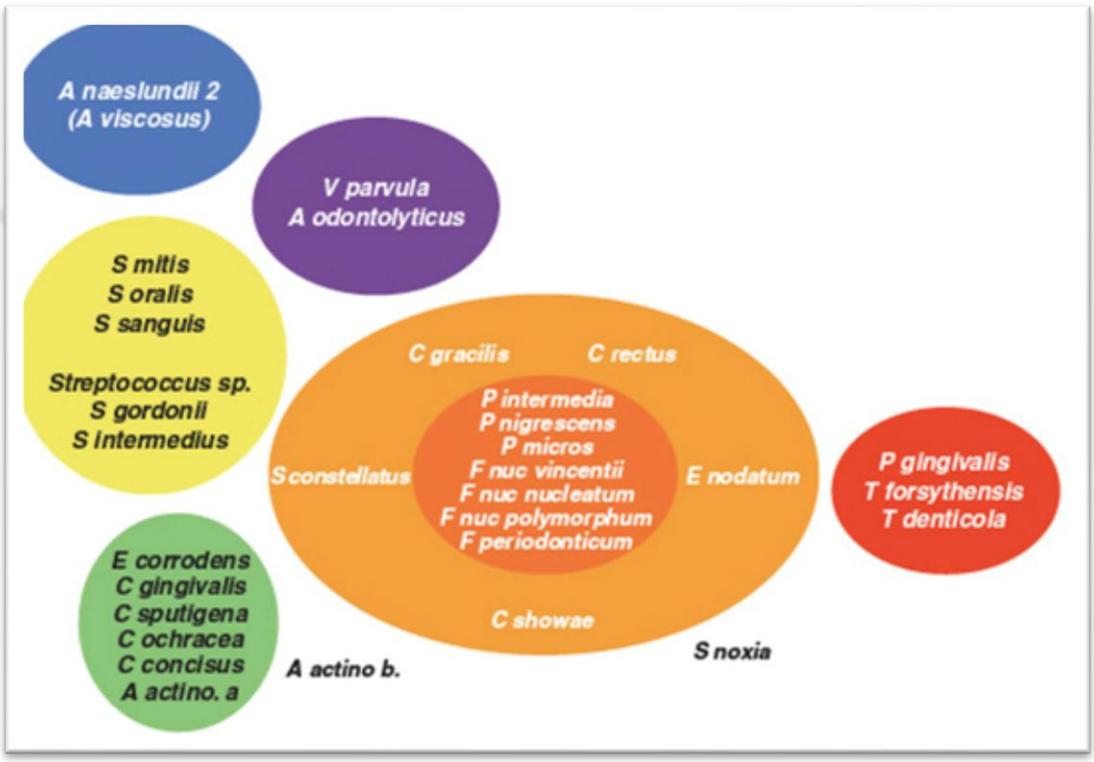

Figure 1. Microbial comities associated with periodontal disease (19).

2.2. Chronic Periodontitis

CP is the most prevalent form of periodontitis, it is a chronic inflammatory disease which effects the tooth and tooth surrounding tissues, leading to tissue destruction as a consequence of the interaction between the subgingival microbiota and the host defenses in susceptible individuals (21). It is characterized as a slowly progressing inflammatory disease, and occurs when; there is a susceptible host, in presence of pathogenic species, and the reduction or absence of so-called "beneficial bacteria" (22). However, it can be modified by systemic and environmental factors (e.g., diabetes mellitus, smoking), which alter the host immune response and leads to a more progressive destruction (17, 23). Therefore it can be said that periodontitis is a disease which is not only determined by what occurs in the oral cavity.

CP is a site-specific disease, and as a result: a surface maintains normal attachment levels whereas the other shows characteristic changes, such as; gingival swelling, redness, loss of stipplings, altered gingival margins, BoP, bone and attachment loss, pocket formation, puss formation, furcation exposure, increase tooth mobility, changes in tooth position, and eventually may lead to tooth loss. (24, 25)

As a result of the site-specific nature, the number of teeth with clinical attachment loss classifies CP into the following types: localized CP when less than 30% of the sites show attachment and bone loss, and generalized CP when 30% or more of the sites show attachment and bone loss (26).

The severity of the disease is based on the amount of clinical attachment loss (CAL); mild when there is 1 mm to 2 mm of CAL, moderate when there is 3 mm to 4 mm of CAL, and severe when there is 5 mm or more of CAL (5).

Even though it could be noted that the host factors such as inheritance, tobacco smoking and various other risk factors is what determines whether the disease is going to develop or not. Bacteria is the principal cause of the initial inflammatory lesion and among them is the red complex bacteria (17), which makes choosing appropriate treatment options quite difficult in the treatment of CP (27).

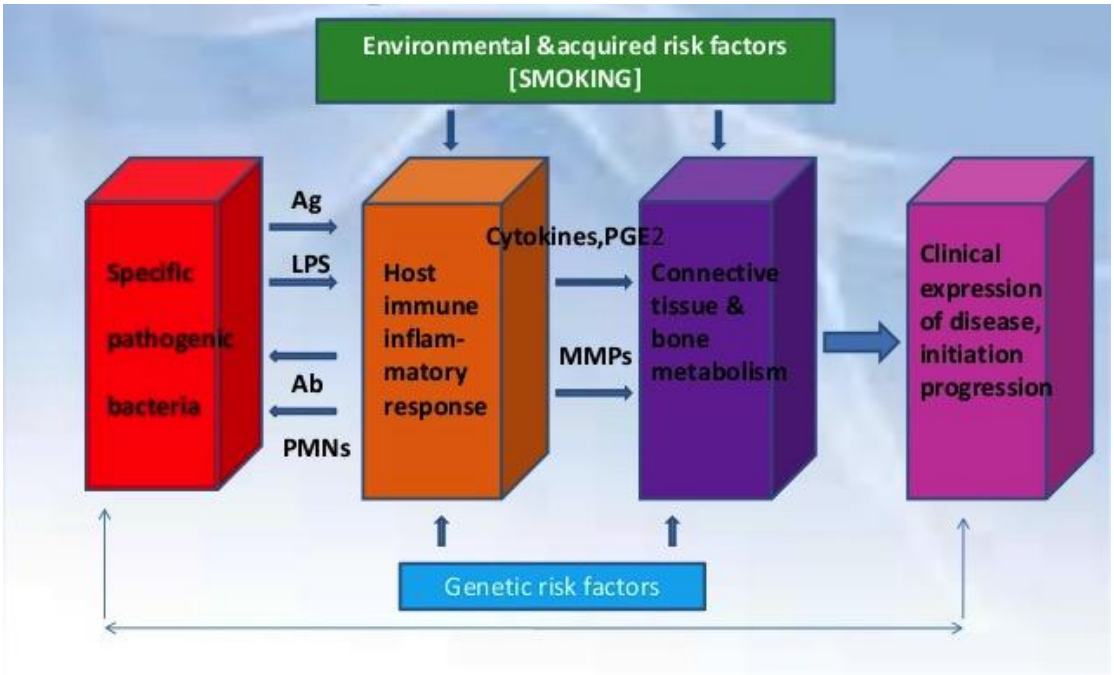

Figure 2. Pathogenesis of human periodontitis (27).

2.3. Initial Periodontal Treatment

Periodontal therapy aims to establish and maintain the health of the periodontium throughout the mouth. Due to the role of microorganisms in the initiation of periodontal disease, mechanical plaque removal is considered the bases of periodontal treatment (28).

Many factors such as tooth morphology, grooves or concavities found on root surfaces, and the presence of enamel extension to furcation in some individuals, provides an ecologic niche for biofilm maturation and subgingival calculus formation. In addition, faulty maintained restorations, fixtures applied without considering the biological width, and carious lesions also do promote plaque retention. Calculus due to its rough surface is able to retain and harbor plaque bacteria and therefore it is considered the most important plaque-retentive factor. The treatment plan for periodontal disease includes four phases. Phase I (non- surgical phase) aims to eliminate the etiologic factors of gingival and periodontal disease. It encompasses SRP, plaque control and oral hygiene instruction (OHI) and is considered as 'gold standard'. In addition, correction of faulty placed restoratives and prosthetic devices, occlusal correction, antimicrobial therapy, applying of minor orthodontic correction are additional steps in this phase, if needed. This phase stops the progression of the disease, and afterwards the patient is placed to Phase IV (maintenance phase). This phase is to preserve the results and prevent recurrence. After observation, if phase I was defined as sufficient then the patient enters phase III (restorative phase) where final restorations, fixed and removal prosthodontics appliances and the response to restorative procedures are evaluated. But if phase I is defined as un-sufficient, then it is considered as preparatory phase for surgical therapy and the patient enters to Phase II (surgical phase), which includes resective, and regenerative surgeries, implant placement, and construction for necessary restorative work (29, 30). The 'need for surgery' outcome measure can be calculated according to Cionca et al. (31) where a site was considered as "in need for surgery" if the probing depth (PD) was ≥ 6 mm or 5 mm and bleeding on probing (BoP) was positive. A tooth was considered in need for surgery if it had at least one site in need for surgery, a patient was considered in need for surgery if at least one tooth was in need for surgery. After all is set down, the patient re-enters to the maintenance phase, and according to his/her needs a re-call time is determined.

Initial periodontal treatment involves supra- and subgingival mechanical debridement and instruction in self-administered oral health measures resulting in reductions in the total microbiota (26). The primary objective of initial periodontal treatment is to disrupt subgingival biofilm and remove bacterial deposits from root surfaces in order to stop further tissue destruction and eliminate or control etiological factors together with creating a microbial shift towards a flora more associated with health. These microbiological changes in turn results in lower levels of inflammation and relative stable periodontal attachment levels (2, 4, 5).

Although a thousand fold reductions in bacteria can be achieved immediately after SRP, no less than a week to months the initial number of bacteria is reached again by pathogens re-colonizating the periodontal pockets (6–8, 32-34), which can be explained by poor hygiene follow up, and the insufficient removal of all deposits due to the limited instrumentation.

Limited instrumentation may be explained by couple of factors, such as (9):

- As PD increases subgingival debridement becomes more difficult (35). Studies have suggested that complete plaque and calculus removal is nearly impossible in pockets exceeding 4 mm in depth for hand instruments (36) and slightly deeper for power-driven instruments (37).
- Key pathogens such as *Aggregatibacter actinomycetemcomitans (A.a)*, *P.g* and *Preoetella intermedia (P.i)* besides the periodontal pocket, they can also be detected in all intraoral niches such as the tongue, tonsils and the mucous membranes (38). The existence of an intraoral translocation (from one niche to another) of periodontal pathogens has been demonstrated (39).
- The capacity of several periodontal pathogens to invade the epithelium or connective tissues or perhaps even the dentinal tubules from which they can regrow (40).
- The presence of morphological variations, such as; differ in tooth morphology, grooves or concavities on root surfaces, and enamel extension to furcation.
- Subgingival instrumentation is not equally effective on all species. Especially *A.a*, and to a lesser extent *P.g*, seem to be quite resistant to subgingival instrumentation, and the degree of their persistence is correlated with a reduced healing response (41).

Due to the multifactorial etiological nature of periodontitis, choosing appropriate treatment options can be quite difficult, and since there is no clear-cut consensus on this subject; many adjunctive treatment approaches have been proposed (42).

Antimicrobial agents, lasers and photodynamic therapy, have been proposed to be used as an adjunctive approaches to non-surgical periodontal therapy, and actually led to temporary improvement of the results. The use of antimicrobial agents has been associated with the increasing levels of bacterial resistance as well as many side effects. In addition, the bacteria within the biofilm are more resistant to antimicrobial agents whereas lasers and photodynamic therapy still need improvements in terms of clinical efficacy (9, 10, 43, 44)**.**

Recently host modulation agents and probiotics have drawn attention to the adjunctive usage of these agents to initial periodontal therapy in the field of periodontology.

2.4. Host Modulation

Host can be defined as "the organism from which a parasite obtains its nourishment". *Modulation* is defined as "the alteration of function or status of something in response to a stimulus or an altered chemical or physical environment" (*Taber's Medical Dictionary,* 2004) (12).

The concept of host modulation was first introduced to dentistry by Williams (45) and Golub et al (46). In 1990, Williams (45) concluded that, "there are compelling data from studies in animals and human trials indicating that pharmacologic agents, that modulate the host responses believed to be involved in the pathogenesis of periodontal destruction, may be efficacious in slowing the progression of periodontitis". In 1992, Golub (46) and colleagues discussed the "host modulation with tetracycline's (TCs) and their chemically modified analogues".

Up to knowledge it is the host that harbors the pathogens, which are responsible for initiating the periodontal disease. Since the theories about the pathogenesis of periodontal disease shifted from it being plaque-associated disease to a more recent hypothesis; that the host's response to bacteria by host-derived enzymes known as the *matrix metalloproteinases* (MMPs), as well as changes in osteoclast activity driven by cytokines and prostanoids are what cause most of the tissue destruction in the periodontium, led to a great influence in the development and improvement of HMT. The elevation in the pro-inflammatory (destructive) mediators (MMPs, prostaglandins (e.g., prostaglandin E_2 [PGE_2]), interleukins (e.g., IL-1α, IL-1β, IL-6), and tumor necrosis factor alpha (TNF-α)) in response to bacterial challenge, are balanced by elevations in the anti-inflammatory (protective) mediators such as the cytokines IL-4 and IL-10, as well as other mediators, such as IL-1ra (receptor antagonist), and tissue inhibitors of metalloproteinases (TIMPs). If the anti-inflammatory mediators are adequate in the response against bacterial challenge, the individual will be disease resistant, otherwise tissue destruction will likely to ensue. Due to the potential of HMTs in down-regulating destructive aspects and up-regulating potential aspects, in combination with SRP that is responsible in reducing the bacterial load, restoration of the balance between health and disease is observed and a direction towards healing is seen. MMPs in addition to cytokines, prostanoids and osteoclasts are the primary target of HMTs (12).

A variety of different drugs have been evaluated as host modulatory agents, including the NSAIDs, Bisphosphonates, TCs, SDD, EMP, GF, BMP, Resolvin, Lipocsin, and Azitromicin.

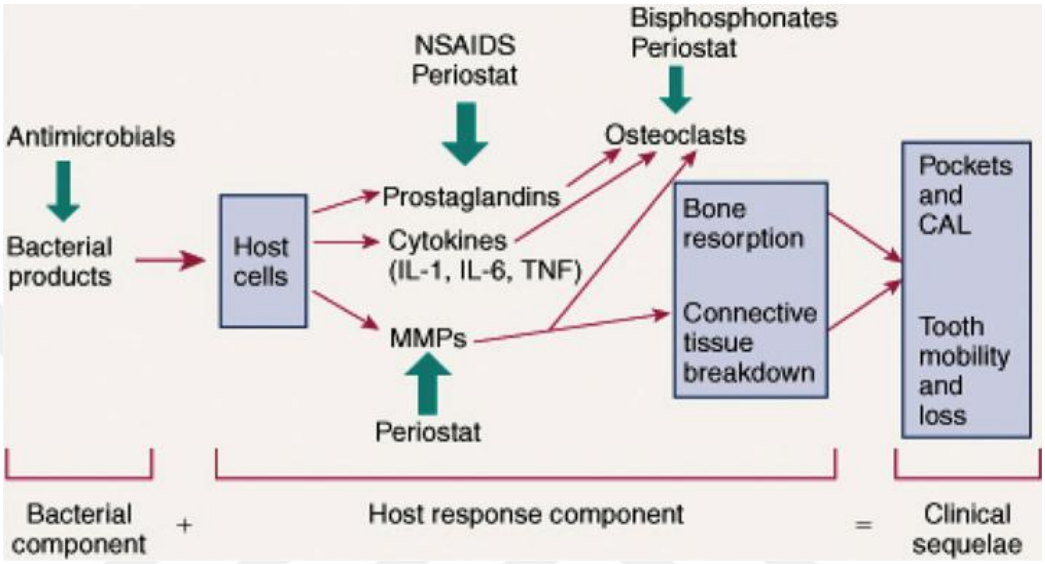

Figure 3. Potential adjunctive therapeutic approaches (12).

2.4.1. Non-steroidal Anti-inflammatory Drugs

NSAIDs inhibit the formation of prostaglandins including PGE_2, which has inhibitory and modulatory effects on the immune response, and thereby reduces tissue inflammation and inhibits osteoclastic activity. Eventually a slower rate of alveolar bone loss is observed (12).

Although it has shown beneficial effects, it is accompanied with serious side effects in long-term usage, which is essential when indicated as adjunct to initial periodontal therapy. In addition to the side effects (gastrointestinal problems, hemorrhage and renal and hepatic impairment), their beneficial affects on the periodontal tissues stop and bone loss rates returns to pre-treatment levels in drug cessation (12).

2.4.2. Bisphosphonate

Bisphosphonates are bone-seeking agents that inhibit bone resorption by disrupting osteoclast activity. Studies have shown, that the usage of bisphosphonates resulted in enhanced alveolar bone status and density (47, 48). Although they have shown promising results, they have been associated with, medicine-related osteonecrosis of the jaw (MRONJ) (49), making them not suitable HMT agent in the treatment of periodontitis.

2.4.3. Sub-Antimicrobial-Dose Doxycycline

SDD is a 20-mg dose of doxycycline and the only systemically administered HMT product approved by the US FDA and accepted by ADA, and recently accepted by Europe and Canada to be used as adjunct to SRP in the treatment of CP (12).

Doxycycline is a semi-synthetic member of the TCs family. TCs have been used in short-term orally administered regimens (10 days – 2 weeks) in traditional doses (100 mg q.d. or b.i.d.) as a broad-spectrum antibiotic. TCs have been used as adjunct to SRP in nonsurgical periodontal treatment, as well as in resective and regenerative surgical procedures to enhance reattachment or even to stimulate new attachment of the supporting tissues and bone formation (50). However, doxycycline's safety profile, pharmacokinetic properties, and ready systemic absorption features made it a drug of choice rather than TCs (51). SDD is chosen as HMT agent in the treatment of periodontitis, due to the effectiveness of doxycycline in down-regulating the activity of MMPs by a variety of synergistic mechanisms, in addition it reduces cytokine levels

and up-regulates collagen production leading to stimulation in osteoblastic activity and new bone formation. It was first designed by Golub et al (51). by two strategies of drug development to suppress connective tissue breakdown including bone loss during periodontitis and other dental and medical diseases using non-antibacterial TCs (50). The first strategy involved the chemical modification of the TCs molecule to deliberately eliminate its antibacterial activity [ie., removal of a chemical side-chain on the TCs molecule, the dimethyl-amino group at carbon-4, which is known to be necessary for the drug's antibacterial activity], but which retained or even enhanced its MMP-inhibitory properties. This allowed the drug to be used as a non-antibiotic TC at both low and high oral doses. As a result of these experiments, they identified another site on the TC molecule, this one responsible for its anti-MMP activity (the calcium and zinc-binding, ß-diketone moiety at carbon-11 and -12), and then developed a series of chemically-modified TCs (ie., the CMTs or COLs) which were therapeutically effective in animal models of various diseases. However, significant side effect such as increased sensitivity to sunburn was observed. As a result, newer compounds are being developed by their group, which are potent inhibitors of MMPs and reducers of the inflammatory mediators, and are expected to be safer.

The second strategy involved systematically reducing the amount of doxycycline formulated in each capsule which, when administered to human subjects in clinical trials, produced blood levels of this drug that were too low (<1 μg/ml; typically 0.25 -0.8 μg/ml) to be effective as an antibiotic. This formulation, once confirmed during clinical trials, contained 20 mg doxycycline per capsule and was administered b.i.d.; this is in contrast to traditional antimicrobial dose doxycycline (ADD) at 100 mg b.i.d, which produces "peak" blood levels of 2-5 μg/ml. This novel, "low-dose" formulation (better known as SDD) reduced the side-effects of systemic antibiotic-dose TC therapy but retained the ability to suppress the tissue-destructive MMPs, to decrease inflammatory mediators (e.g., IL-1ß), and to reduce diagnostic biomarkers of bone resorption in the periodontal pocket. Additionally studies have shown that the usage of SDD showed no differences, neither in the composition nor in the resistance level of the oral flora (52, 53). More recent studies also showed no overgrowth of opportunistic pathogens, such as *Candida*, in the oral cavity, gastrointestinal system, or genitourinary system (54), which makes it a HMT agent and not an antibiotic.

2.4.3.1. Safety, Indications and Contra-Indications

The rationale for using SDD must be clearly explained to the patient. By discussing the etiology of periodontal disease, the available treatment options, and the anticipated outcomes.

Indication for SDD usage (12);

- In the management of CP,
- In the management of aggressive periodontitis,
- In the management of general health problems,
- As adjunct in periodontal surgery.

Contra-indications to SDD usage;

- Gingivitis or periodontal abscess,
- When antibiotic is indicated,
- Patients with a history of allergy or hypersensitivity to tetracycline,
- Pregnant or lactating women,
- Children younger than 12 years old.

Doxycycline at antibiotic doses (≥100 mg) is associated with adverse effects, including photosensitivity, hypersensitivity reactions, nausea, vomiting, and esophageal irritation. However, in the clinical trials of SDD (20-mg dose), it was reported that the drug was well tolerated, and the profile of unwanted effects was virtually identical in the SDD and placebo groups (13, 55-57). Additionally no evidence of developing antibiotic resistance of the microflora after 2 years of continuous use was observed (13, 52, 53, 58, 59). Therefore the drug appears to be well tolerated, with a very low incidence of adverse effects.

2.4.3.2. SDD in General Health

Over the past decades, SDD has been proposed in patients with medical disorders where excessive amounts of MMPs and inflammatory mediators play role (50),

- SDD significantly reduced the severity of inflammatory lesions in patients with acne and rosacea (erythema patches on the face, as well as pustules and papules, and “spider-like” veins on the nose and cheeks) (60).
- Reduction in the blisters and ulcers in the oral mucosa of patients with mucous membrane pemphigoid (61).

- In combination with the anti-inflammatory effects it was found to be effective in the treatment of rheumatoid arthritis (62).
- Reduction in the elevated levels of MMPs in the urine and clinical and physiological improvement in lung function were observed in patients with lymphangioleiomyomatosis, which eventually led to improvement in life quality (63).
- Significant reduction in circulating levels of HgA1c in patients with type I and II diabetes (64).
    a) It was also evaluated in the treatment of post-menopausal bone loss and resulted in; decreased progression of periodontal breakdown, and reduced loss of alveolar bone,
    b) Reduced levels of local biochemical biomarkers and mediators of periodontal breakdown including decreased leukocyte type collagenase (MMP-8),
    c) Reduced levels of systemic biomarkers of bone resorption in the circulation indicated a reduced risk, in these postmenopausal women, of conversion of mild skeletal bone loss (osteopenia) into the more severe form of bone loss, osteoporosis (65).
- Reduction in the biomarkers of systemic inflammation strongly associated with cardiovascular disease including the tissue-destructive proteinase, MMP-9, the long-term pro-inflammatory cytokine, IL-6, and the acute-phase protein, C-reactive protein. In addition reduced risk for a fatal heart attack were observed in patients with acute coronary syndromes which includes a history of myocardial infarction, atherosclerosis, and blood chemistry indicating cardiac damage (66).

2.4.3.3. Sub-Antimicrobial Dose Doxycycline In Periodontal Disease

Several studies regarding the usage of SDD as adjunct to SRP have been performed in 3,6 and 9 months consumption period. Most of these studies aimed to evaluate the adjunctive effect of SDD on biochemical biomarkers (55, 67-72), however only few studies investigated the effects of SDD clinically and microbiologically.

Walker et al. (53) aimed to evaluate whether the SDD exerted antimicrobial effects on the microflora or not in a split-mouth placebo-controlled study design. 76 patients were divided into two groups, SDD and placebo. After baseline sample collection, SRP was performed on two quadrants (either left or right) and the remaining two quadrants were left untreated. The samples were retaken after 3,6 and 9 months of treatment and after 3 months of no treatment. As a result both groups showed statistically significant reduction in the proportions of spirochetes and motile rods and increase in the coccoid forms when compared to baseline. It was concluded that the obtained microbial differences is due to the anti-collagenase and anti-inflammatory properties of SDD rather than its antimicrobial effect.

Novak et al. (56) evaluated the effect of SDD in the management of severe generalized periodontitis. 30 patients were divided into 2 groups and randomly received SDD or placebo for a period of 6 months adjunct to SRP. SRP was conducted once a week for a month and periodontal condition was recorded at baseline, months 1, 3, 5.25, and 8,25. Maintenance therapy was performed at 3, 5.25 and 8.25 months for both groups. As a result they found superior clinical outcomes in favor of the SDD group in all evaluated period, nearly %40 of 237 pockets > 7 mm were reduced by > 4 mm and %55 were reduced by > 3 mm. SRP in combination with SDD was found more effective than a placebo in preventing further increase in probing depth.

Lee et al. (73) evaluated the safety of SDD when used as adjunct to SRP in CP patients. 41 patients were divided into 2 groups randomly and received either SDD or placebo for a period of 2 weeks. Clinical (PD and CAL), microbiological and biochemical (MMP-8, MMP-13) parameters were evaluated. The effects of SDD on the periodontal flora were assessed by using dark-field of microscopic and culture method. As a result, clinical parameters showed a significant improvement in the test group. The dark-field analyses showed a reduction in spirochetes and motil-rods, and an increase in the proportions of cocci and non-motile rods in both treatment groups, however the differences between groups were consider not significant. On the other hand culture analysis showed a decrease in anaerobes and black-pigmented bacteria in both treatment

groups. MMP-8 and MMP-13 mean percentage average was found significantly higher in the placebo group. The study concluded that the usage of SDD in the management of CP was found effective and safe.

In addition to all the beneficial effects that were achieved when used as adjunct to SRP in the treatment of CP several studies have shown that SDD can be also used in patients with aggressive periodontitis who are being treated nonsurgically. Furthermore, emerging studies have supported efficacy of SDD as an adjunct to periodontal surgery (74). SDD may also be of benefit in cases that are refractory to treatment, as well as in patients with risk factors such as smoking, diabetes, osteoporosis/osteopenia, genetic susceptibility, and in whom the treatment response might be limited (12).

The reason for at least 3 months of SDD usage is due to the outcomes of the study conducted by Caton and Ryan (75), where SDD was used for a period of 1 month. It was concluded that cessation of SDD administration resulted in rapid rebound of collagenase activity to placebo levels, suggesting that a 1-month treatment regimen with this host modulation agent was insufficient to produce a long-term benefit.

2.5. Probiotics

2.5.1. History of Probiotics

The term ‘‘probiotic’’ is a relatively new word and is currently used to name bacteria with beneficial effects for humans and animals. The usage of probiotics goes back as early as 1877 when Pasteur and his associate, Joubert noted that the growth of anthrax bacilli in cocultures with ‘common bacilli’ (probably Escherichia coli) was suppressed. They commented that ‘these facts perhaps justify the highest hopes for therapeutics’ (24). In the early 1900’s when the Nobel Prize winner Ilya Metchnikof a Ukrainian bacteriologist, stated that ‘the change of diet and ingesting lactic acid which is found in dietary products displace pathological intestinal microbiota and thereby ‘replace the harmful microbes by useful microbes’ (76, 77). In 1953 Kollath (78) explained it as “Probiotika are active substances that are essential for a healthy development of life” and in 1965 Lilly & Stillwell (79) introduced it as ‘‘Substances produced by micro-organisms which promote the growth of other micro-organisms’’. Since 1965, several definitions for probiotics have been proposed (Parker 1974 (80), Fuller 1989 (81), Havenaar & Huis In’t Veld 1992 (82), Schaafsma 1996 (83), Naidu et al. 1999 (84), Salminen et al. 1999 (85) Schrezenmeir & de Vrese 2001 (86)) and finally in 2002 World Health Organization (WHO), and the Food and Agriculture Organization (FAO) defined it “ live microorganisms which, when administered in adequate amounts, confer a health benefit on the host’’ (http://www.who.int/foodsafety/fs_management/en/probiotic_guidelines.pdf).

Table 1. Definition of Probiotics.

Year	Definition	Reference
1953	Probiotika are active substances that are essential for a healthy development of life	Kollath (78)
1965	Substances produced by microorganisms that promote the growth of other microorganisms	Lilly & Stillwell (79)
1974	Organisms and substances that contribute to intestinal microbial balance	Parker (80)
1989	A live microbial feed supplement that beneficially affects the host animal by improving its intestinal microbial balance	Fuller (81)
1992	A viable monoculture or mixed-culture of microorganisms that, when applied to animal or human, beneficially affects the host by improving the properties of the indigenous microflora	Havennaar & Huis In't Veld (82)
1996	Living microorganisms that, upon ingestion in certain numbers, exert health benefits beyond inherent basic nutrition	Schaafsma (83)
1999	A microbial dietary adjuvant that beneficially affects the host physiology by modulating mucosal and systemic immunity, as well as by improving nutritional and microbial balance in the intestinal tract	Naidu et al. (84)
1999	Probiotics are microbial cell preparations or components of microbial cells that have a beneficial effect on the health and well-being of the host	Salminen et al. (85)
2001	A preparation of, or a product containing, viable, defined microorganisms in sufficient numbers, which alter the microflora (by implantation or colonization) in a compartment of the host and as such exert beneficial health effects in this host	Schrezenmeir & de Vrese (86)

2.5.2. Prebiotics

Prebiotics are defined as ‘not digestible food ingredients that beneficially affect the host by selectively stimulating the growth and ⁄ or activity of one or a limited number of bacterial species already established in the colon, and thus in effect improve host health’. These prebiotics include insulin, fructo-oligosaccharides, galacto-oligosaccharides and lactulose. Prebiotics as well probiotics aim to improve host health by modulating intestinal flora, with a difference in mechanism of action. In some cases such as gastro-intestinal application, prebiotics is beneficial for probiotics and this is known as symbiotic concept. Symbiotics are defined as ‘mixtures of probiotics and prebiotics that beneficially affect the host by improving the survival and implantation of live microbial dietary supplements in the gastro- intestinal tract of the host’ (22, 87). However the use of prebiotics to effect probiotic strain to remain longer in the mouth still needs to be evaluated.

2.5.3. Replacement Therapy

Replacement therapy is another term also appears in the literatures which is also called ‘bacteriotherapy’ or ‘bacterial interference’. It is most likely to be confused with probiotics, due to that both of them use live bacteria for the prevention or treatment of infectious disease, but distinguished by slight differences.

Review of the literature reveals only a few numbers of studies in terms of replacement therapy applied in periodontology. The first one by Teughels et al. (43) serves as a pioneer study in this context. In an *in vivo* beagle dog model for periodontitis these authors explored that the subgingival application of beneficial bacteria interferes or retards the recolonization of periodontal pockets after SRP.

Table 2. Differences between 'replacement' and 'probiotic' therapy (22).

Replacement therapy	Probiotic therapy
Effector strain is not ingested and is applied directly on the site of infection	Probiotics are generally used as dietary supplements
Colonization of the site by the effector strain is essential	Probiotics are able to exert a beneficial effect without permanently colonizing the site
Involves dramatic and long-term change in the indigenous microbiota	Rarely a dramatic and long-term microbiological change
Directed at displacing or preventing colonization of a pathogen	
Has a minimal immunological impact	Exerts beneficial effects by influencing the immune system

2.5.4. Classification of Probiotics

There are a number of different organisms that can be classified as probiotics (77). Most commonly used probiotics are *Lactobacillus* and *Bifidobacterium* strains, in addition other strains as *Escherichia, Enterococcus, Bacillus* and *Streptococcus* have been documented (88).

Table 3. Names of microorganisms used as probiotics (88).

Lactobacillus sps.	*Bifidobacterium sps.*	*Streptococcus sps.*	*Sacchoromyces sps.*	*Others*
L.acidophilus *L.casei* *(rhamnosus)* *L.fermentum* *L.gasseri* *L.johnsonii L.lactis* *L.paracasei* *L.planrarum* *L.reuteri* *L.sallivarius* *L.bularicus*	*B. bifidum* *B.breve* *B.lactis* *B.longum* *B.infantis* *B.adolescentis*	*S.thermophilus* *S. salivarius subsp. thermophilus*	*S.boulardii*	*Bacillus cereus* *Escherichia coli* *Enterococcus* *Propioni-bacterium freudenreichii*

2.5.5. Application of Probiotics

Probiotics at the present time are provided in four basic ways and can be administered by choosing one of the ways (89):

- Inoculated into prebiotic fibers,
- As a culture concentrate added to a beverage or food (fruit juice),
- Inoculated into a milk-based food (milk, cheese, kefir, biodrink),
- As concentrated and dried cells packaged as dietary supplements (non-dietary products such as capsule, powder, gelatin tablets).

Different formulation of the products and their spread around the world are listed in table 4.

Table 4. Major probiotic formulas and products around the world (89).

Strain	Present in product	Country produced
B. bifidum	Infant formula	Turkey
B. breve	Drink	Japan
B. lactis	Infant formula Research Drink	Israel Switzerland South Africa Chile
B. lactis HN019	Research	New Zealand
B. longum	Infant formula	Turkey
B. longum SBT-2928	Milk	Japan
B. longum BB536	Milk	Japan
B. spp	Drink	UK
L. acidophilus	Yogurt Drink Yogurt drink	Chile, USA UK Austria
L. acidophilus 5	Yogurt drink	UK
L. acidophilus 7	Yogurt	Austria
L. acidophilus Lat 11/83	Drink	Russia

L. acidophilus NCFB 1748	Research	Denmark
L. acidophilus SBT-2062	Milk	Japan
L. bulgaricus	Milk	France, Austria
L. casei DN-114 001	Drink	France, Austria
L. casei Shirota	Drink	Argentina, Australia, Belgium, Brazil, Brunei, China, Germany, France, Hong Kong, Indonesia, Japan, Korea, Luxembourg, Mexico, Netherlands, Philippines, Singapore, Taiwan, Thailand, Uruguay, UK, USA
L. casei	Drink Yogurt Kefir	USA USA USA, Austria
L. helveticus	Milk Drink	Finland Iceland
L. johnsonii La1	Yogurt	Switzerland, Germany, Japan, Austria
L. lactis L1A	Yogurt	Sweden
L. plantarum	Kefir	USA
L. plantarum 299v	Fruit drink Ice cream Recovery drink Oat mixture	Sweden Sweden Sweden Sweden
L. plantarum JI:1	Research	Sweden
L. reuteri	Infant formula Cheese Milk Yogurt Yogurt drink Ice cream Fruit drink Tablet Straw	Israel Spain, Portugal, Finland Japan, Finland USA,Finland UK Finland Finland

	Yogurt	Australia, Papua New Guinea, Indonesia, Finland, Latvia, Estonia, Croatia, South Korea, Bosnia-Herzegovina, Slovenia, Ecuador, Israel, Italy, Netherlands, Japan, Norway, Switzerland Australia, Finland, Sweden, Croatia, Bosnia-Herzegovina, Slovenia, Ecuador, Uruguay, Netherlands, Taiwan, Norway
L. rhamnosus ATCC53103(LGG)		
	Yogurt drink	Finland, Sweden UAE, Israel, Italy Germany, Portugal, Japan, Iceland, Greenland, Spain, Estonia, Ireland, Israel, South Korea Finland
	Fruit yogurt Milk Milk drink Fruit drink	
L. rhamnosus	Drink	Finland, Sweden, Chile, South Africa
L. rhamnosus LB21	Yogurt	Sweden
L. rhamnosus 271	Drink	Sweden
L. salivarius U CC 118	Research	Ireland
L. rhamnosus VTTE-97800	Research	Finland
S. salivarius K12	Lozenge	New Zealand
S. thermophiles	Drink Yogurt drink Infant formula	France, Austria Austria Turkey
E. faecium	Yogurt	Denmark

2.5.6. General Features and Mechanism of Action

The mechanism of action of probiotics is related to their ability to compete with pathogenic microorganism for adhesion sites, to antagonize these pathogens or to modulate the host's immune response (90).

The selection criteria for probiotics are (91):

- Adhesion and colonization of the human body. Adhesion increases the contact of bacteria with host surface, thus facilitating further probiotic activity,
- Increase the non-specific and specific immune response of the host,
- Survival and resistant to human defense mechanisms during transition (e.g. tolerance to acids (low pH) in the mouth and stomach, and tolerance to bile in the upper intestine),
- Safety to the macro-organism.

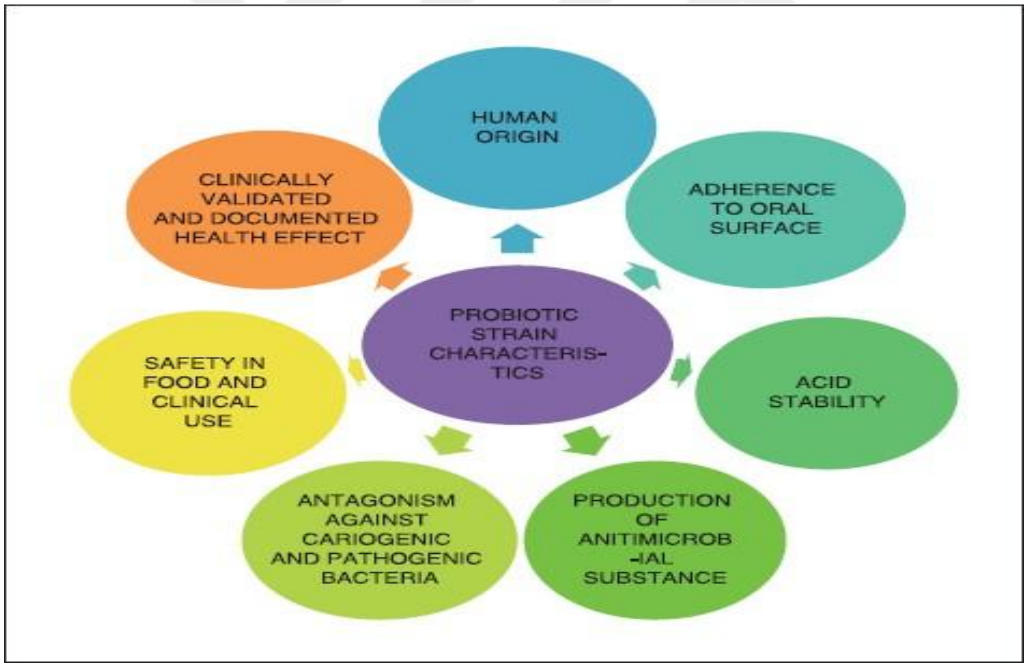

Figure 4. Selection criteria for probiotics (91).

Mechanisms of probiotics in the oral cavity may be either direct interaction with dental plaque or indirect by modulating host defenses (92).

Direct interactions include inhibition of specific pathogens by:

- Involvement in binding of oral microorganisms to proteins (biofilm formation).
- Action on plaque formation and on its complex ecosystem by competing and intervening with bacteria-to-bacteria attachments.
- Involvement in metabolism of substrates (competing with substrates available).
- Production of chemicals that inhibit oral bacteria (antimicrobial substances).

Indirect interactions include effects on the host response such as:

- Inhibition of collagenases and reduction of inflammation-associated molecules.
- Induction of expression of cytoprotective proteins on host cell surfaces.
- Modulation of pro-inflammatory pathways induced by pathogens.
- Prevention of cytokine-induced apotosis.
- Modulation of host immune response.

2.5.7. Safety of Probiotics

The safety (defined as absence of clinical adverse reactions such as nausea, stool characteristics, vomiting, bacteremia, flatulence or abdominal symptoms) of *L. reuteri* has been documented in several human clinical trials in healthy adults, children, infants and neonates as well as in immuno-suppressed HIV-positive volunteers. Two of these studies also showed good *in vivo* survival in humans confirmed by enumeration of *L. reuteri* in faecal samples. From these and other studies it was concluded that a dose of 10^8 cfu/day was well tolerated, safe and efficacious in man (77, 93).

2.5.8. *Lactobacillus reuteri*

Lactobacilli are divided into three groups according to the metabolic way they fallow to ferment carbohydrates.

1) The obligate homofermentative group (e.g., *L. acidophilus*, *L. delbrueckii*, *L. helveticus*, *L. salivarius*) possesses a fructose diphosphate (FDP) adolase pathway dictating a glycolytic conversion of sugars primarily into lactic acid.
2) The facultative heterofermentative group (e.g., *L. casei, L. curvatus, L. plantarum, L. sake, L. rhamnosus*) can use either this FDP adolase pathway to ferment certain sugars, or they can induce the phosphoketolase pathway to ferment other sugars.
3) The obligate heterofermentative group (e.g., *L. brevis, L. buchneri, L. fermentum, L. reuteri*) that has only the phosphoketolase-based option (94).

Lactobacilli are thought to be involved in the maintenance of the microbiota, and are present in the gastrointestinal and vaginal tract in healthy individuals (95). *L.reuteri* is a specific biotype of *Lactobacillus fermentum* and was first isolated by Lerche and Reuter in 1962. In 1980, Kandler et al. (96) described this biotype as *L. reuteri,* a new subspecies of heterofermentative lactobacilli. *L.reuteri* is a rod shaped, gram-positive, non-spore forming, non-motile, and does not require anaerobic conditions for growth and is normally cultivated in oxygen-limited atmospheres. *L. reuteri* strains are fastidious and rely on the availability of easily fermentable sugars, amino acids, vitamins and nucleotides. If these factors are provided, the organisms grow very fast (93). *L. reuteri* ATCC 55730 (and its daughter strain DSM 17938) has been demonstrated in several studies to have probiotic properties (97-101). It is considered to be one of the few true indigenous *Lactobacillus* species in man. It has also been reported that *L.reuteri* produce compounds that exhibits antagonistic activity, which are also considered broad-spectrum antimicrobials, i.e. reuterin (102) and reutericyclin (103). These antimicrobials are water-soluble, effective over a wide pH, and are resistant to proteolytic and lypolytic enzyms (103, 104).

2.5.9. Probiotics in General Health

Various beneficial health effects resulted from the consumption of probiotic bacteria have been reported (105, 106). Although the mechanism of action is not fully understood, still the evidence suggests that probiotics can influence various diseases positively. However, these organisms are already produced in the diary products and they are rarely implicated in human infections, therefore they are categorized as 'Generally Regarded As Safe' by the U.S FDA (107).

- Prevention and/or reduction of duration and complaints of rotavirus-induced or antibiotic-associated diarrhea as well as alleviation of complaints due to lactose intolerance.
- Reduction of the concentration of cancer-promoting enzymes and/or putrefactive (bacterial) metabolites in the gut.
- Prevention and alleviation of unspecific and irregular complaints of the gastrointestinal tracts in healthy people.
- Beneficial effects on microbial aberrancies, inflammation and other complaints in connection with: inflammatory diseases of the gastrointestinal tract, Helicobacter pylori infection or bacterial overgrowth.
- Normalization of passing stool and stool consistency in subjects suffering from constipation or an irritable colon.
- Prevention or alleviation of allergies and atopic diseases in infants.
- Cholesterol lowering properties in humans, by causing direct assimilation of lipids, convert them into other metabolites and end products, which affects synthesis of cholesterol (108).
- Symptomatic improvement inpatient with irritable bowel syndrome (IBS) (109).
- Benefit effects to infants. Probiotics provide a positive impact on the immune system by stimulating antibody production, reduce the incidence of diarrhea, exerts a preventive effect on atopic eczema even beyond infancy, increase weight, length and occipital circumference, increase absorption of minerals thus help to increase bone density (109).
- Prevention of respiratory tract infections (common cold, influenza) and other infectious diseases as well as treatment of urogenital infections. Insufficient or at most preliminary evidence exists with respect to cancer prevention, the so-called hypocholesterolaemic effect, prevention or therapy of ischemic heart diseases or amelioration of autoimmune diseases (e.g. arthritis).

2.5.10. Probiotics in Oral Health

The oral cavity is an ecological open growth system, and is connected to the middle ear through the Eustachian tube, the nasopharynx, the larynx, the tonsils and eventually the gastrointestinal tract, it is conceivable that these anatomically related regions can influence or can be influenced by the oral microbial ecology. Thus the oral cavity is considered an important area of treatment (22).

The microbiota of the oral cavity contains a wide range of bacterial species, and it is estimated that more than thousands of these species can colonize the oral cavity (110, 111), which makes it a unique and complex structure. *Streptococcus and Lactobacillus* species, *Fusobacterium, Bacteroides, Porphyromonas, Prevotella, Haemophilus, Eubacterium, Bifidobacterium, Neisseria, Veillonella, Capnocytophaga, Peptostreptococcus, Staphylococcus, Proprionibacterium, Cornyebacterium, Actinomyces* and *Treponema* are the predominant organisms in the oral cavity (110). These commensal bacteria has a beneficiary effect on oral tissues by preventing the colonization of exogenous pathogens, promotes normal development of host cell structure and function, ensure normal development of the immune system, and down regulates the immune response. However with the development of biofilm, expression of resident phenotypes; and the development of food webs and interactions such as quorum- sensing to communicate with each other, leads the resident bacteria to gain significant advantages such as protection from the host defenses and antimicrobial agents (112-115). The most common disease regarding oral health is dental caries and periodontal disease, due the imbalance accruing when the dental plaque initiates the process and host responses, insufficiently (87).

Recently potential application of probiotics for oral health has attracted researchers to investigate oral probiotics, suggesting that probiotics could be useful in preventing and treating oral infections, such as dental caries (116, 117), periodontal disease (118), halitosis (119), and *Candia albicans* infections (120). In order to achieve probiotic effect in the oral cavity, it is essential for the microorganism to adhere to saliva-coated surfaces, to colonize and grow in the mouth, and to inhibit oral pathogens. Therefore, pattern of adhesion of different probiotic strains to oral epithelial cells have been investigated (77).

2.5.10.1. Probiotics and Periodontal Disease

Periodontal diseases are an end result of host response to the complex action of a group of periodontal bacteria, predominantly Gram-negative anaerobes. Since the primary etiological factors for the development of periodontal disease are bacteria present in the biofilm, efforts for disease prevention and treatment are mainly focused on pathogen reduction and strengthening of the epithelial barrier (91). Although, SRP results in a shift towards a less pathogenic microbiota, the results are only temporary, and the re-colonization of the microbiota with more aggressive pathogens occurs within weeks to months (6, 28, 32, 121).

The recent knowledge is that the presence of periodontal pathogens could be regulated by means of antagonistic interactions by probiotic bacteria (77). The first studies in the field of periodontology on probiotics started with experimental gingivitis studies.

Staab et al. (122) evaluated the effect of a probiotic milk drink on gingival health and the development of experimental gingivitis. Fifty volunteer students were included in a parallel-designed non- blinded study. The test group drank a probiotic drink once a day; where as the control group did not receive any product to drink. After 8 weeks, individual mechanical plaque control was interrupted for 96 h. Clinical and immunological parameters were measured at baseline and after 8 weeks and again 96 h later. The authors were not seen any significant differences between the groups in terms of the clinical parameters. In the test group, some of the immunological parameters were detected significantly lower after the intake of the probiotic milk drink. The authors reported that the consumption of probiotic containing milk had benefits on the periodontal health in non-immunocompromised patients.

Twetman et al. (123) investigated the effect of a chewing gum containing *L.reuteri* on gingival inflammation and the levels of selected inflammatory mediators in gingival crevicular fluid (GCF). The study was designed as double-blinded, placebo-controlled clinical trial. Forty-two healthy adults with moderate levels of gingival inflammation were recruited and were randomly divided into 3 groups: Group A/P; received one active and one placebo gum daily, Group A/A; received two active gums, and Group P/P; received two placebo gums. The chewing gums contained two strains of Lactobacillus reuteri: ATCC 55730 and ATCC PTA 5289 ($1X10^8$ CFU/gum, respectively). The subjects were instructed to chew the gums for 10 min over the course of two weeks. BoP and GCF sampling were done at baseline and after 1, 2, and 4

weeks. The levels of IL-1b, TNF-a, IL-6, IL-8 and IL-10 were detected using luminex technology and multiplex immunoassay kits. Although BoP values and GCF volume improved in all groups during the chewing period, only A/A and A/P groups showed statistically significant results. The levels of TNF-α and IL-8 decreased significantly in Group A/A compared to baseline after 1 and 2 weeks, respectively. However IL-1BETA levels showed no significant decrease at all. As a result, the usage of probiotics reduced the pro-inflammatory cytokines in the GCF, suggesting that it might be used as a therapeutic agent in gingival inflammation conditions.

Krasse et al. (118) aimed to evaluate the effect of *L. reuteri* in the treatment of gingivitis together with the influence of the probiotic on plaque and the *lactobacilli* population in saliva. The study was design was placebo-controlled, double-blinded with duration of two weeks. Fifty-nine patients with moderate to severe gingivitis were divided into three groups, group II and I were given one of two different *L. reuteri* formulations at a dose of 2×10^8 CFU per day, and group III received placebo. Gingival index and plaque index were measured; in addition saliva samples were collected for *lactobacilli* determination at baseline and week 2. As a result, all three groups showed significant decrease in gingival index measurements, while plaque index measurement showed a significant decrease only in one of the active group. At the end of the study period both active groups showed *L. reuteri* colonization, suggesting that the probiotic usage was effective in reducing both gingival inflammation and plaque in patients with moderate to severe gingivitis.

Iniesta et al. (124) evaluated the effects of *L. reuteri* on the oral microbiota in gingivitis patients. 40 volunteers with gingivitis were divided into 2 groups, and the *L. reuteri-containing* or placebo lozenges were administered once a day for 8 weeks. Clinical and microbiological samples were collected. Unstimulated saliva and subgingival samples were collected and analyzed by culture and PCR. As a result, no significant differences were detected between the groups in terms of clinical variables However, the test group showed significant reduction in saliva total anaerobic counts after 4 weeks and counts of *Prevotella intermedia* (*P.i)* levels after 8 weeks. The authors concluded that *L. reuteri* containing tablets resulted in a reduction in the number of selected periodontal pathogens in the subgingival microbiota, without having an impact on the clinical parameters.

Koll-Klais et al. (125) reported that including a concentration of 10^8 CFU/ml probiotic strains to periodontal dressings diminished the number of most frequently isolated periodontal pathogens: *Bacteroides sp.*, *Actinomyces sp*., and *S. intermedius*, and also *Candida albican*s. As a result, the authors registered that a flora resident with *lactobacilli,* inhibits the growth of *P.g* and *P.i.* In addition the application of the periodontal dressing that is consisted of collagen and *L. casei* results in a 10 to 12 month remission period after periodontal treatment.

Ishikawa et al. (126) observed that *L. salivarius* TI 2711 (LS 1) was able to kill *P. g, P. i* and *P. nigrescens* after 6-12 hours of co-culturing together. The authors also evaluated the ability of LS 1 to displace periodontopathogens in an *in vivo* study. 76 volunteers were included in an 8-week study period and no pretreatment was performed. The subjects were divided randomly into 3 groups. Group I and II received *L. salivarius* TI 2711 in tablets either 2×10^7 cfu/day or 1×10^8 cfu/day, five times a day**,** and group III did not receive any product. As a result, black-pigmented anaerobic rods, in the saliva decreased significantly in both probiotic groups, whereas the numbers of whole bacteria, *S. mutans* and *lactobacilli* did not show any significant changes. The authors suggested that probiotic agents against periodontal pathogens could be a useful agent.

Shimauchi et al. (127) aimed to evaluate the effect of probiotic intervention using *lactobacilli* on the periodontal condition of volunteers without severe periodontitis. The subjects were divided into test and control groups and subdivided into smokers and non-smokers. Freeze-dried *L. salivarius* WB21 (WB21)- containing tablets or a placebo were administered to subjects. The study was designed as a double blind, randomized, placebo controlled clinical trial. A total of 66 subjects were randomly assigned to receive tablets containing WB21 (6.7×10^8 CFU) with xylitol or xylitol alone (placebo) three times a day for 8 weeks. Clinical parameters and whole saliva samples were obtained at baseline, 4 weeks, and at the end of 8 weeks. Salivary lactoferrin levels were measured by enzyme-linked immune-sorbent assay (ELISA). *Lactobacilli* in saliva and plaque samples were detected by semi-quantitative real-time quantitative polymerase chain reaction (RT-PCR) using 16S rRNA primers. The authors reported significant improvements in clinical parameters in both groups after an 8-week intervention. Current smokers in the test group showed a significantly greater improvement in plaque index and PD from baseline when compared with those in the

placebo group. Salivary lactoferrin level was also significantly decreased in the test group in smokers. The authors concluded that probiotics could be useful in the improvement/ maintenance of oral health in subjects at a high risk of periodontal disease.

In the study conducted by Mayanagi et al. (128), they evaluated whether the oral administration of *lactobacilli* could change the bacterial population in supra/subgingival plaque in a randomized double-blind placebo controlled clinical trial. Sixty-six healthy subjects without severe periodontitis were allocated into two groups to receive either *lactobacilli* (2.01x 109 CFU/day of *L. salivarius* WB21 and xylitol) or placebo (only xylitol) over a 8 week follow-up period. The authors suggested that oral administration of probiotic *lactobacilli* decreased significantly the numerical sum of the five selected periodontopathogenic bacteria including *A.a, P.i, P.g, T.d,* and *T. f.*

Tsubura et al. (129) evaluated the effect of *Bacillus subtilis* containing mouth rinse (E-300) when compared to Neosteline green (NG) in patients with CP. Fifty-four patients were divided into two groups. Group I received E-300 and Group II received NG mouth rinse twice a day for a period 30 days. Clinical and microbiological samples were obtained at baseline and day 30. As a result, test group (Group I) showed significantly reduced periodontal pathogen levels and an improvement in gingival index score while probing pocket depth and bleeding on probing showed small improvements. The authors reported that *Bacillus subtilis* was an appropriate mouth rinse for patients with periodontitis.

In a recent study by Laleman et al. (130) they evaluated the adjunctive effects of a *Streptococcus oralis* KJ3, *Streptococcus uberis* KJ2 and *Streptococcus rattus* JH145 containing probiotic tablet in combination with SRP. Forty-eight patients were included in the double blind, placebo-controlled clinical trial and were divided into SRP + Probiotic and SRP + Placebo groups. Tablets were consumed twice a day for a period of 12 weeks. PD (primary outcome measure), BoP and relative attachment level (RAL) were measured at baseline and week 12 and 24. At baseline, 4, 8, 12 and 24 weeks, microbiological sampling was performed and plaque and gingival indices were recorded. As a result there was a significant ($p<0.05$) improvement at week 12 and 24 evaluation period in both groups. However, no significant intergroup differences could be detected at any time point, except from the % of sites with plaque that were significantly lower in the probiotic group at the 24-week evaluation. Additionally, at week 12 salivary *P.i* counts were significantly lower in the probiotic group. No

differences were detected when comparing the adjunctive use of a placebo or the investigated streptococci containing probiotic tablet after SRP.

Up to date, there are only 5 studies evaluating the efficacy of the probiotic *L. reuteri* in the treatment of periodontal disease. The first one of these studies, which also serves as a pioneer is, the study conducted by Vivekananda et al. (131). They evaluated the effects of *L. reuteri* alone and in combination with SRP. Thirty systemically healthy individuals with CP were included in a split mouth, randomized, placebo-controlled clinical trial. SRP was performed as a "split-mouth" design, where only two quadrants were treated (either right or left) and the remaining two quadrants were left untreated. Four groups occur due to the split mouth design of the study (SRP + Probiotic, Probiotic, SRP + placebo, placebo), SRP was performed on day 0; the participants received a toothbrush, toothpaste, and brushing instructions. *L. reuteri* containing lozenges ($1x10^8$ CFU DSM17938+$1x10^8$ CFU ATCC PTA 5289) or the corresponding placebo lozenges was taken twice a day from day 21 to day 42. Clinical parameters as plaque index (PI), gingival index (GI), gingival bleeding index (GBI), PD and CAL in addition microbiological levels of the pathogens *A.a*, *Pg*, and *P.i* were evaluated. At day 42 all treatment modalities showed significant reduction in all investigated clinical parameters and the amount of the reduction was detected as SRP + Probiotic, Probiotic, SRP + Placebo, Placebo, respectively. Probiotic, either alone or following SRP, reduced *Aa*, *Pi*, and *Pg*. However the SRP + placebo combination did not significantly affect the levels of the pathogens. As a result, the authors confirmed the plaque inhibition, anti-inflammatory, and antimicrobial effects of *L. reuteri* probiotic and that it can be recommended during non-surgical therapy and the maintenance phase of periodontal treatment.

Teughels et al. (132) evaluated the clinical and microbiogical effects of *L. reuteri* containing probiotic lozenges as an adjunct to SRP in CP patiens. Thirty patients were included in a randomized, placebo-controlled study. All patients received one-stage full-mouth disinfection and were randomly divided into groups; Group I: SRP + Probiotic and Group II: SRP + Placebo, respectively). Group I received *L. reuteri* containing lozenges whereas group II received sucralose containing placebo lozenges. The lozenges were administered twice a day for a period of 12 weeks. At week 12, all clinical parameters were significantly reduced in both groups; while there was significantly more PD reduction and attachment gain in moderate and deep pockets in addition *P.g* levels in Group I. The researchers suggested that oral administration of *L.*

reuteri lozenges could be a useful adjunct to SRP in CP.

In a study conducted by Vicario et al. (133) clinical effect of *L.reuteri* in the treatment of initial to moderate CP patients was evaluated in a 30-day period. In addition patient compliance and side effects were also evaluated. Twenty individuals were included in this double-blinded, placebo-controlled, randomized clinical trial. No SRP was applied and subjects were randomly assigned to receive tablets containing Probiotic or placebo once a day for 30 days. Clinical parameters were measured at baseline and 30 days post- treatment. At the end of day 30, test group demonstrated a statistically significant reduction in all the clinical parameters while the control group did not show any statistically significant changes.

Tekce et al. (134) evaluated the clinical and microbiological effect of *L. rueteri* containing lozenges as an adjunct to initial periodontal therapy in CP patients and to detect *L. reuteri* colonization in periodontal pockets over 12 month follow up period. 40 patients were included in a double-blinded placebo control study and were randomly divided into two groups (Group I= SRP + Probiotic, and Group II= SRP + Placebo). After SRP was performed, Probiotic or placebo lozenges were administrated for a period of 21 days. Clinical parameters (PI, GI, BoP, PD and clinical attachment gain) and microbiological sampling were performed at baseline and days, 21, 90, 180 and 360. Statistically significant improvements in all clinical parameters were observed within each group in terms of PI, GI, BoP, PD and attachment gain. Parallel to the clinical findings, microbiological parameters as TVC and proportions of obligate anaerobes within each group were significantly reduced after days 21, 90, and 180 for both groups except day 360. Within the limits of the study it was concluded that, *L. reuteri* containing lozenges might be an adjunctive useful agent for retarding recolonization and the improvement of periodontal health.

Ince et al. (135) evaluated the clinical and biochemical efficacy of *L. reuteri* containing lozenges as an adjunct to SRP in CP patients. A total of 40 patients were included in a double-blind placebo-controlled study and were randomly divided into probiotic and placebo groups. Individuals consumed the lozenges for a period of 21 days. Clinical and biochemical samples were obtained at baseline and days 21, 90, 180, and 360. At the end of the study period, statistically significant improvements in all evaluated parameters were observed within each group. GCF MMP-8 and TIMP-1 levels revealed significant differences in the probiotic group at all time intervals except day 360. However at day 360, the significance on the clinical parameters continued to

exist.

However, there is not yet any true evidence on the effect of probiotic therapy on periodontal disease, and the effect of the ingested probiotics needs further investigation. Therefore, the objective of this study was to evaluate the effects of *L. reuteri* containing lozenges in comparison to SDD containing tablets on the clinical and microbiological parameters over a 3-month period in CP patients.

The null-hypothesis of this study was that neither the clinical profile nor the microbiological parameters would differ between all three groups.

3.MATERIALS AND METHODS

3.1. Patient Selection and Inclusion Criteria

Forty-five systemically healthy CP patients aged between 35-50 years, who were seeking for periodontal care or referred for periodontal treatment at Yeditepe University, Faculty of Dentistry Department of Periodontology were screened for this study.

Patient selection criteria were as follow:

1) CP patients with radio-graphically detected horizontal bone loss.
2) Presence of at least 2 teeth, having one approximal site with PD of 5-7 mm and GI of ≥ 2 in each quadrant
3) No use of probiotic supplements
4) No periodontal or antimicrobial treatment within 6 months
5) No systemic disease
6) No smoking
7) No pregnancy and lactating
8) No adverse reactions to lactose or fermented milk products
9) No allergies to tetracycline products
10) No use of any aluminium, calcium, iron, magnesium, and zinc containing products.

Patients fulfilling the inclusion criteria had a detailed explanation on the purpose of the study. Patients willing to participate in this study signed a written informed consent (Appendix 1).

The present double blind, parallel, controlled and randomized clinical trial was conducted according to the guidelines of Helsinki Declaration of Human Rights.

3.2. Probiotic Containing Lozenges Under Investigation

The probiotic lozenges consisted of two strains of *L. reuteri* at a dose of $2x10^8$ CFU *(*DSM17938 and ATCC PTA5289). The placebo lozenges[1] consisted of suclarose with no active probiotic strains. Both probiotic and placebo lozenges[2] were identical in shape, texture and taste and therefore could not be discriminated from each other. Patients were asked twice a day after tooth brushing to place the lozenge in the moth and allowing it to dissolve. Patients were also instructed not to consume any food or beverages for one hour after the use of the lozenge and not to use any probiotic containing products during the course of the study.

3.3. Doxycycline Hyclate Containing Tablets

SDD is a 20-mg dose of doxycycline (Periostat®). SDD capsule is taken twice a day, at least one hour before meal on an empty stomach. SDD must be taken with water and must not eat for at least one to two hours after consuming the capsule. Exceeding the recommended dosage may result in an increased incidence of side effects including the development of resistant microorganisms.

3.4. Treatment Groups

Group I. (SRP+ Probiotic) (Test group n=15):

Patients in this group consumed probiotic (Prodentis®) and sucralose containing lozenges as adjunct to SRP. Each subject was instructed to take the lozenges for a period of 3 months twice a day after tooth brushing by placing it in the mouth and allowing it to dissolve.

Group II. (SRP+SDD) (Test group n=15):

Tablets containing 20 mg of doxycycline hyclate (Periostat®) were consumed as adjuncts to SRP. Patients were instructed to take the tablets twice a day on an empty stomach for a period of 3 months.

Group III. (SRP+ Placebo) (Control group n= 15):

Only sucralose containing lozenges were consumed as adjuncts to SRP. Each subject was instructed to take the lozenges for a period of 3 months twice a day after tooth brushing by placing it in the mouth and allowing it to dissolve.

[1] BioGaia ProDentis, Stockholm, Sweden
[2] BioGaia Suclarose Lozenges, Stockholm, Sweden

3.5. Randomization and Study Design

This study design was randomized, double-blinded, placebo-controlled clinical trial. The patients were divided into three treatment groups according to a computer-based randomization table. Patient's personal information such as, name, surname, address, age, systemic history, and the usage and dosage of any drugs if consumed were all noted prior to the study.

Table 5. Randomization table.[3]

Group I (SRP + probiotic)														
P 1	**P 2**	**P 3**	**P 4**	**P 5**	**P 6**	**P 7**	**P 8**	**P 9**	**P 10**	**P 11**	**P 12**	**P 13**	**P 14**	**P 15**
31	15	42	34	3	25	11	7	24	41	16	12	6	33	21
Group II (SRP + SDD)														
P 1	**P 2**	**P 3**	**P 4**	**P 5**	**P 6**	**P 7**	**P 8**	**P 9**	**P 10**	**P 11**	**P 12**	**P 13**	**P 14**	**P 15**
39	32	26	22	35	18	27	8	13	45	5	43	17	28	23
Group III (SRP + Placebo)														
P 1	**P 2**	**P 3**	**P 4**	**P 5**	**P 6**	**P 7**	**P 8**	**P 9**	**P 10**	**P 11**	**P 12**	**P 13**	**P 14**	**P 15**
30	36	19	14	10	44	40	9	4	2	37	29	1	20	38

P: Patient number

[3] www.randomizer.org/Copyright 1997-2011 by Geoffrey C. Urbaniak and Scott plous

A week prior to the experimental period each patient was given OHI. Dental models were used for a brief explanation, and the patients were asked to brush their teeth (Bass method) at least twice a day in combination with interdental devices. Then the patients were randomly divided into SRP + SDD, SRP+ probiotic and SRP + Placebo treatment groups.

At baseline, intraoral photographs and microbiological samples were obtained by the usage of paper-point. In addition, clinical measurements including PI, GI, PD, RAL and BoP, were obtained. SRP was conducted under local anesthesia by ultrasonic devises[4] and gracey curettes[5]. Afterwards tablets/lozenges administration was begun, and SRP was repeated a week after. At day 90 administrations was stopped and all measurements including photographs, clinic and microbiologic samplings were repeated.

[4] Piezon® OEM Built- in Kit, EMS, Switzerland

[5] Gracey, SG 3/4, 5/6, 7/8, 11/12, 13 / 14 mini-five, SAS $^{3}/_{4}$,Hu–Friedy, USA

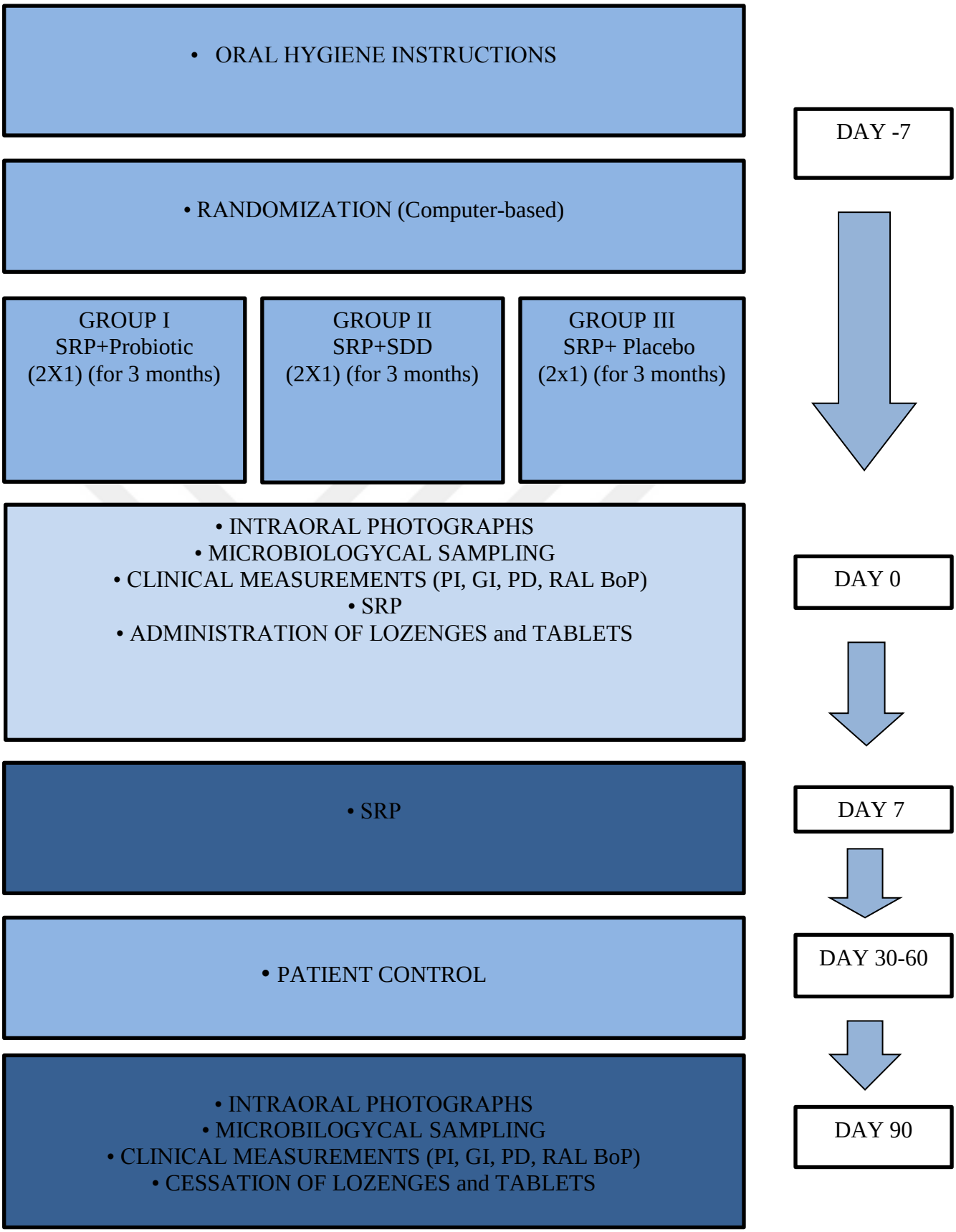

Figure 5. Flowchart of the study.

3.6. Adverse Events and Patient Compliance

At baseline patients were given the tablets/lozenges (doxycycline hyclate, probiotic or placebo). At day 7, 30, 60 and day 90, patients were asked for compliance or any adverse events that they might have noticed. Additionally the usage of any drugs was questioned.

3.7. Clinical Indices and Measurements

Individually prepared acrylic occlusal stents were used for each patient and helped to place the probe properly at each measurement appointment and eventually reduce the errors associated with probe[6] placement. Six grooves were placed on the stents so that the measurements would include mesio-buccal, disto-buccal, buccal, lingual/palatinal, disto-lingual/palatinal and mesio-lingual/palatinal. All measurements were performed at baseline and day 90, and recorded by the same calibrated examiner using a 0.4 mm diameter 15 mm calibrated periodontal probe.

3.7.1. Plaque Index

Teeth were isolated with cotton rolls and dried with air syringe. The dental plaque was evaluated by placing the probe on 4-tooth surface (mesio-buccal, buccal, disto-buccal and lingual/palatinal) and scores between 0-3 were given for each point (136).

Scoring was made as follows:

0- No microbial dental plaque in the gingival area.

1- A film of microbial dental plaque adhering to the free gingival margin and adjacent area, recognized only by running a probe across the tooth surfaces.

2- Moderate accumulation of soft deposits within the gingival pocket and on the gingival margin and/or adjacent tooth surfaces that can be seen by naked eye.

3- Abundance of soft matter within the gingival pocket and/or on the gingival margin and adjacent tooth surface.

[6] University of North Carolina PCPUNC 15, Hu-Friedy Ins Co, Chicago, IL, USA

3.7.2. Gingival Index

Periodontal probe was used to assess the bleeding potential of the tissues from 4 tooth surfaces (mesio-buccal papilla, buccal margin, disto-buccal papilla and lingual/palatinal margin) and scores between 0-3 were given for each point (137).

Scoring was made as follows:

0 – Normal gingiva

1 – Mild inflammation, slight change in color, slight edema, no BOP

2 – Moderate inflammation, redness, edema, ulcerations; tendency to spontaneous bleeding.

3 – Severe inflammation, ulceration and spontaneous bleeding.

3.7.3. Probing Depth

Full mouth PD was measured at 6 sites per tooth (mesio- buccal, buccal, disto-buccal, mesio- lingual/palatal, lingual/palatal, disto-lingual/palatal). By using the grooves on the occlusal stands the probe was inserted parallel to the axis of the tooth into the periodontal pocket. The distance between the gingival margin and the bottom of the periodontal pocket is considered the PD.

3.7.4. Attachment Gain

Full mouth RAL, which is the distance between the occlusal stent margin, and the bottom of the periodontal pocket, was measured from 6 surfaces of the tooth (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual and disto-lingual).

3.7.5. Bleeding on Probing

BoP was assessed from six aspects of the teeth (mesio-buccal, buccal, disto-buccal, mesio-lingual/palatal, lingual/palatal, disto-lingual/palatal), and was determined by the presence or absence of bleeding up to 30 sec. after PD was recorded (138).

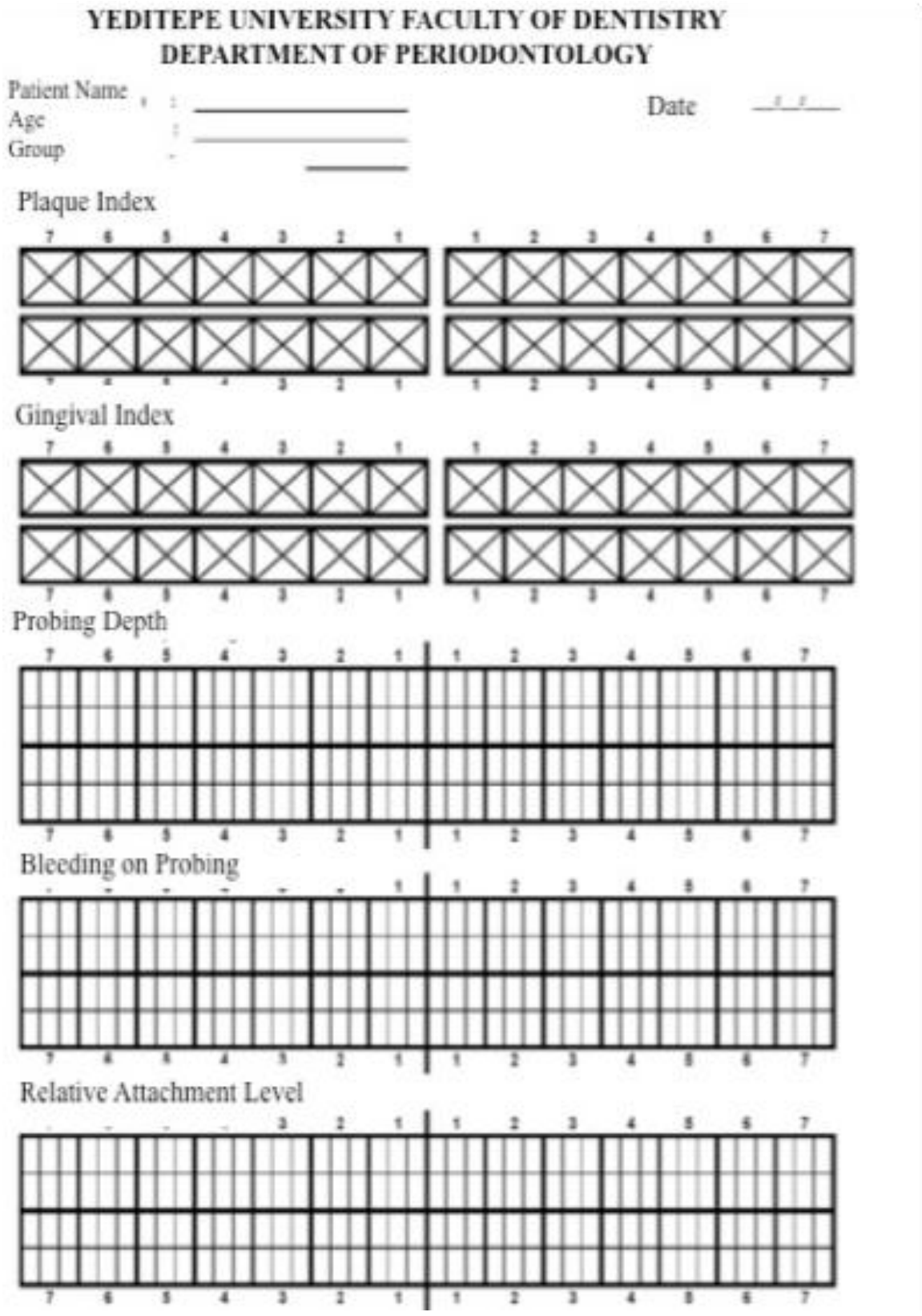

Figure 6. Data sheet.

3.8. Microbiological Procedures

3.8.1. Sample Collection and Microbiologic Culturing

For microbial sampling, 2 single rooted- teeth in each quadrant with approximal PD 5 - 7 mm and GI ≥2 were selected. Samples were taken at baseline and re-taken from the same teeth at day 90.

After cleaning the sample area with cotton rolls and taking care in order to avoid contamination, the samples were obtained and pooled before the microbial analysis.

Paper-points[7] were used for the microbiological sampling. They were inserted in the pockets of the selected tooth until resistance was felt (Figure 7). After 10 seconds, the paper points (Figure 8) were transferred to 4,5 ml Phosphate Buffered Saline[8] and dispersed using a vortex mixer at maximal setting for 30 seconds, and then serially tenfold diluted. From each dilutions (10^{-1}, 10^{-2},.... 10^{-5}) two portions of 0,1 ml was taken and plated separately onto tryptic soy agar[9] medium supplemented with %5 defibrinated sheep blood, 0.0005% hemin[10] and 0.00005% menadione[11] .The first tripc soy agar plate was incubated at 37˚C for 7 to 10 days in Gas Jars[12], while the other plate was incubated at 37˚C in %10 CO_2 for 4 days.

TVC was determined as the total number of bacterial colonies on plates anaerobically incubated (Figure 9, 10). All the microbiologic data was transformed into colony forming units/milliliter (CFU/ml) of transport medium. In addition, obligate anaerobic bacteria was calculated as the TVC minus the total counts of colonies on plates incubated in 10% CO_2 condition and was expressed as a percentage of TVC. Microbial samples were analyzed by culturing and TVC and proportions of obligate anaerobic bacteria were determined.

[7] # 25/30, DiaDent, Almere, The Netherlands
[8] Phosphate buffered saline tablets, Chalbiochem, Merck KGaA, Darmstadt, Germany
[9] Oxoid Ltd, Basingstoke, Hampshire, England
[10] Sigma-Aldrich Chemie GmbH, Steinheim, Germany
[11] Sigma-Aldrich Chemie GmbH, Steinheim, Germany
[12] AnaeroGen kit, Oxoid Ltd, Basingstoke, Hampshire, England

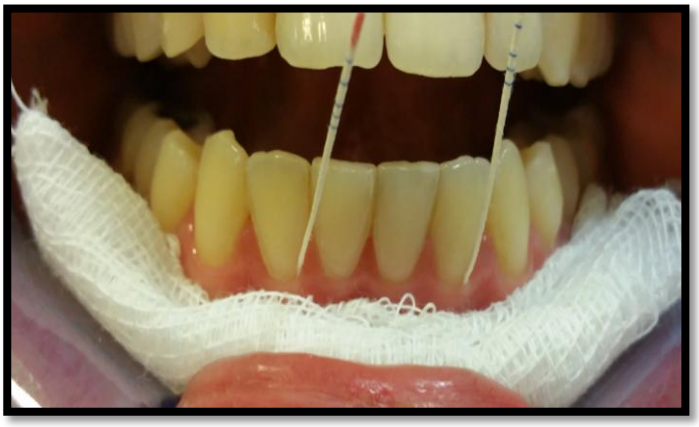

Figure 7. Subgingival plaque sampling.

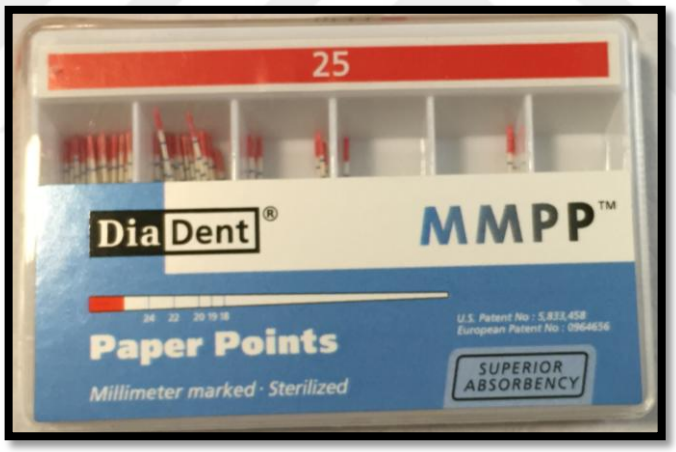

Figure 8. Paper-points used for sampling.

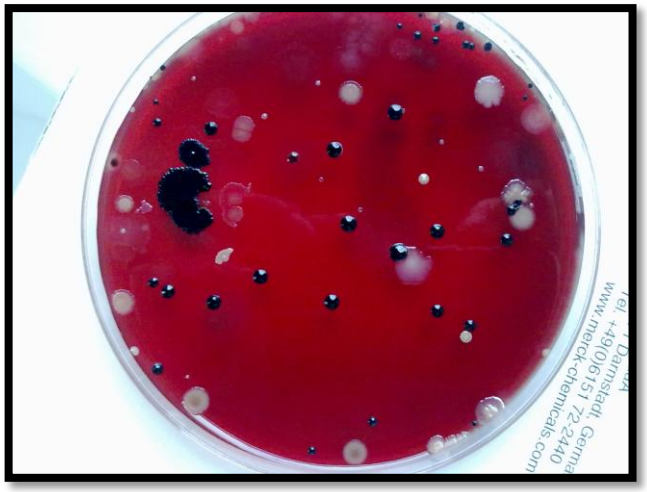

Figure 9. Total Viable Count (TVC(x10^5 CFU/ml)).

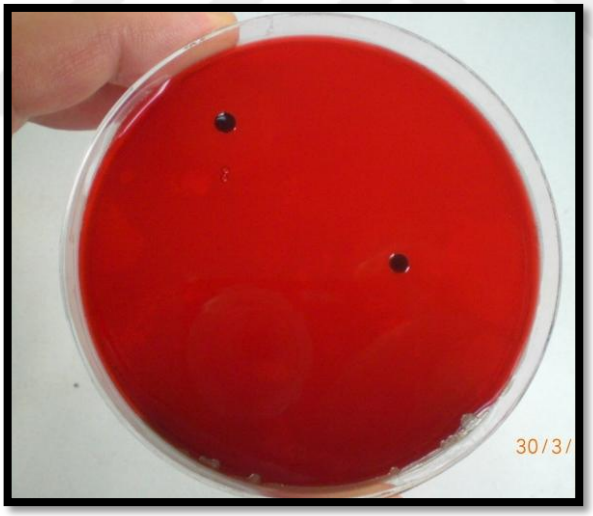

Figure 10. Proportions of Obligate Anaerobic Bacteria in TVC.

3.9. Statistical Analysis

The sample size was calculated for the primary outcome variable, PD reduction, based on the method described by Vivekananda et al. (2010). According to the results of the power analysis, a sample size of seven subjects for each group would yield an 80% statistical power at $\beta = 0.20$ and $\alpha = 0.05$ to detect $\Delta = 0.82$ with a standard deviation (SD) of 0.5. For all statistical evaluations, the patient was used as the unit of measurement. Data analysis was performed for full-mouth PI, GI, BoP, PD, RAL, and TVC measurements and for the proportions of obligate anaerobic bacteria using a statistical package (NCSS 2007 & PASS 2008 Statistical Software, USA). The normal distribution of the outcome measures was evaluated using the Shapiro wilks test. The balancing of the groups by age and gender was tested using Student's t-test and the chi-square test, respectively. Quantitative data were recorded as the mean value of the SD for the PI, GI, BoP, PD, the attachment gain and the proportions of obligate anaerobic bacteria. The median (min–max) values for TVC were also calculated. The paired sample test was used for intra-group comparison of the clinical parameters and proportions of obligate anaerobes, whereas the Wilcoxon sign test was used for the TVC values. One-way ANOVA test was used for the inter-group comparisons of mean differences of clinical parameters and proportions of obligate anaerobes (%) whereas Kruskal-Wallis test was used for the TVC values. Tukey test was used to evaluate the comparisons of the clinical parameters and proportions of obligate anaerobes in pairs, whereas Mann–Whitney U-test was used for the evaluation of TVC values in pairs. Statistical significance was set as $p<0.05$.

4. RESULTS

4.1. Demographic and Baseline Data

A total of 45 systemically healthy, CP patients, 24 males and 21 females, aged between 35-50 years were included in this study. Baseline clinical and microbiological parameters were similar in all three groups (p>0.05) (Table 6). All subjects completed the study period. Intraoral photographs (day 0, 90) and panaromic radiograph one of the representative case from each group are shown in Figures 11.a-c, 12.a-c and 13.a-c.

Table 6. Baseline data of the patients in the treatment groups.

	GROUP I SRP+ PROBIOTIC MEAN ± SD	**GROUP II SRP+SDD MEAN ± SD**	**GROUP III SRP+PLACEBO MEAN ± SD**	**p**
AGE[+]	38 ± 6.49	40.27 ±6.33	40.00 ± 6.57	0.989
GENDER (M/F)[++]	9/6	7/8	8/7	0.765
PI[+]	2.36 ± 0.35	2.47±0.2	2.27 ± 0.3	0.507
GI[+]	2.15 ± 0.17	2.3±0.11	2.2 ± 0.38	0.574
BoP(%)[+]	86.5 ± 13.5	89.01 ± 3.4	89.5 ± 3.67	0.801
PD (mm)[+]	5.19 ± 0.61	5.59±0.21	5.13 ± 1.05	0.486
T.V.C (x10^5 CFU/ml)[+++]	41.00(35-43)	33.5(25-42)	42.00(14-81)	0.086
OBLIGATE ANAEROBES[++] **(%)**	50.08 ± 4.63	48.60 ± 4.49	46.46 ± 3.95	0.177

[+]One way ANOVA, [++]Chi-square test, [+++]Mann Whitney U test, p<0.05, PI: Plaque index, GI: Gingival index, BoP: Bleeding on Probing, PD: Probing Depth, TVC: Total Viable Count

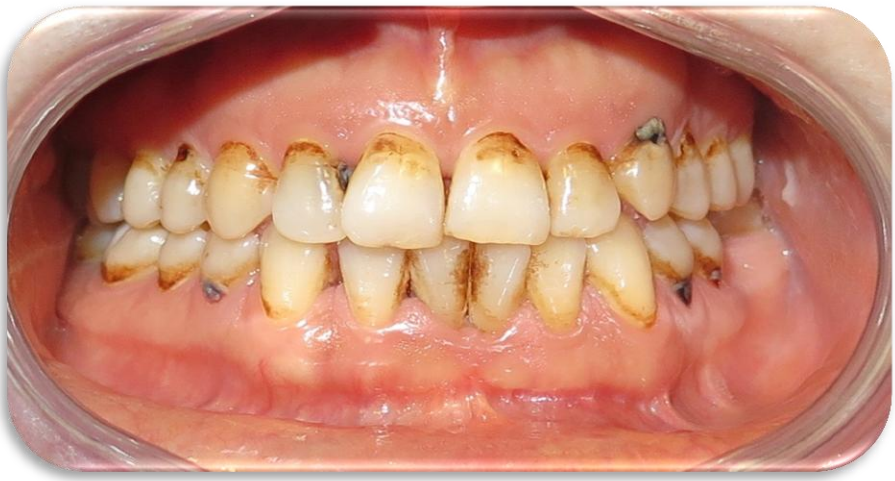

Figure 11.a. Intraoral photograph of a representative case from the SRP + Probiotic group at day 0.

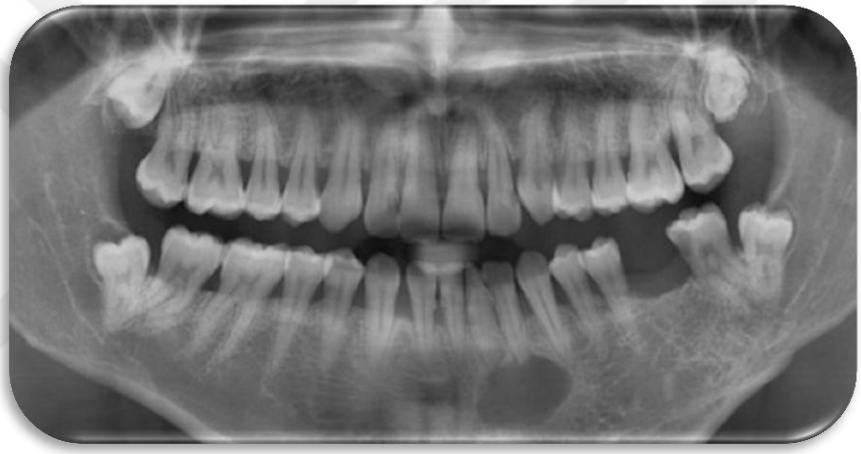

Figure 11.b. Intraoral periapical radiograph of a representative case from the SRP + Probiotic group.

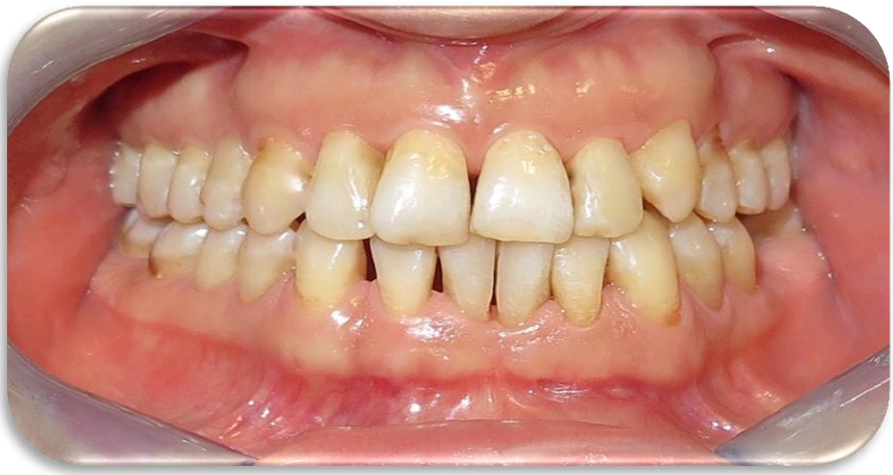

Figure 11.c. Intraoral photograph of a representative case from the SRP+ Probiotic group at day 90.

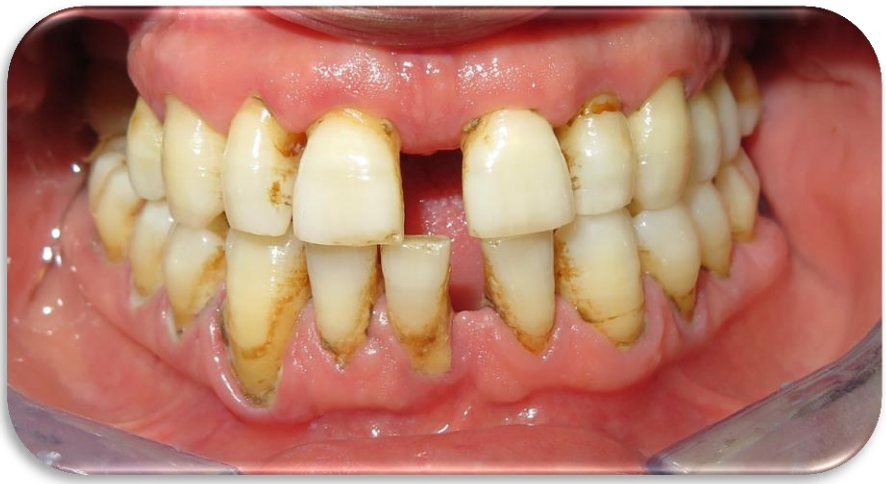

Figure 12.a. Intraoral photograph of a representative case from the SRP + SDD group at day 0.

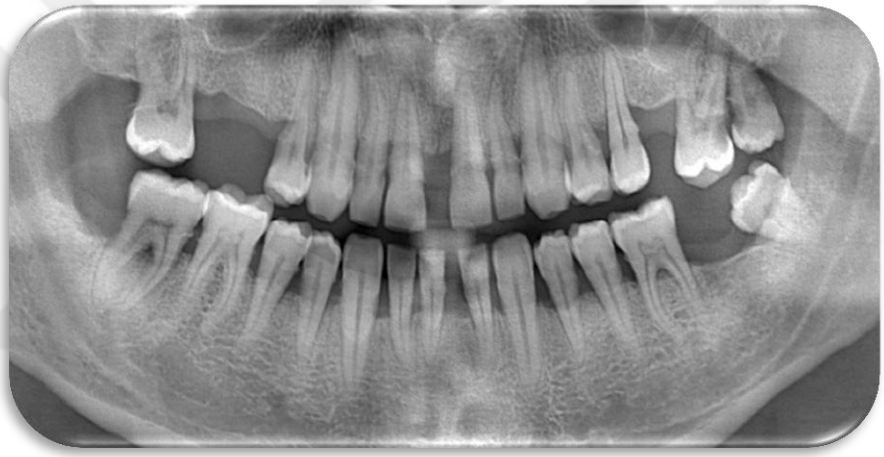

Figure 12.b. Intraoral periapical radiograph of a representative case from the SRP + SDD group.

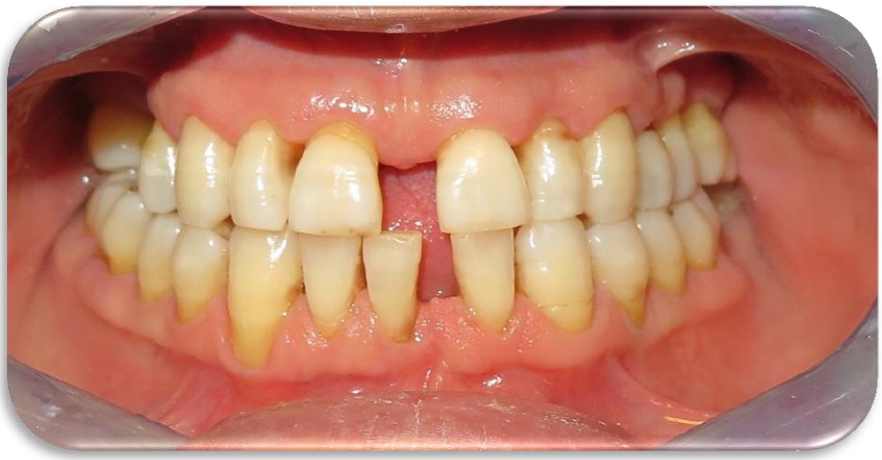

Figure 12.c. Intraoral photograph of a representative case from the SRP + SDD group at day 90.

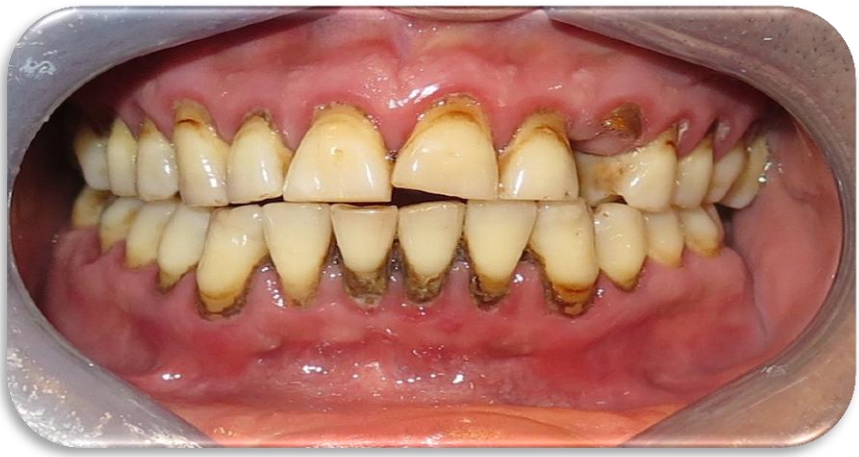

Figure 13.a. Intraoral photograph of a representative case from the SRP+ Placebo group at day 0.

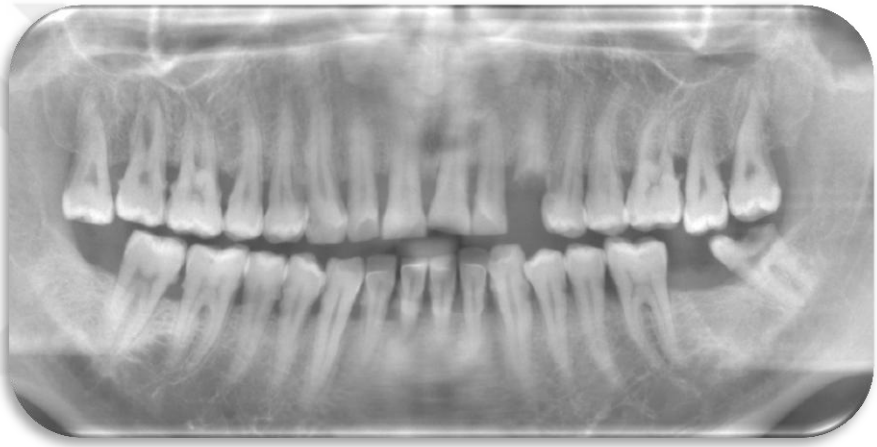

Figure 13.b. Intraoral periapical radiographs of a representative case from the SRP + Placebo group.

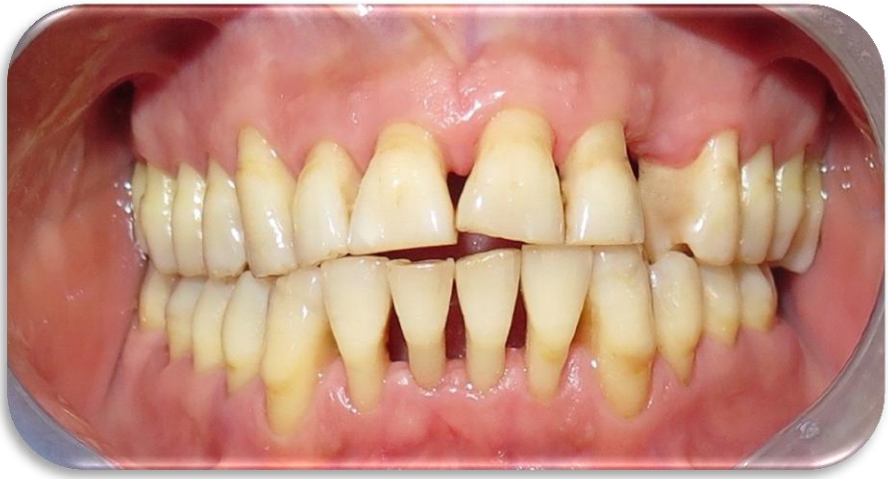

Figure 13.c. Intraoral photograph of a representativecase from the SRP + Placebo group at day 90.

4.2. Clinical Measurements

The mean PI, GI, PD, RAL and BoP, values for baseline and day 90 of all three groups are presented in (Table 6-10).

4.2.1. Plaque Index

No significant differences were detected between groups at baseline in PI values (Table 6). PI values were detected as 2.36±0.35 and 0.55±0.19 in SRP + Probiotic group, 2.47±0.2 and 0.62±0.16 in SRP + SDD group, and 2.27±0.3 and 1.25±0.18 in SRP + Placebo group, at days 0 and 90 respectively. Mean differences of PI values were detected as 1.81±0.4, 1.84±0.06 and 1.30±0.46 between days 0-90, in SRP + Probiotic, SRP + SDD and SRP + Placebo groups, respectively (Table 7-10). Inter-group comparisons of mean differences of PI values revealed statistically significant results in favor of SRP + Probiotic and SRP + SDD group compared to SRP + Placebo group between days 0-90 ($p=0.004$; $p= 0.003$, respectively), however no significant difference was detected between SRP + Probiotic and SRP + SDD group ($p=0.986$) (Table 7-10).

4.2.2. Gingival Index

No significant differences were detected between groups at baseline in GI values (Table 6). GI values were detected as 2.15±0.17 and 0.6±0.23 in SRP + Probiotic group, 2.3±0.11 and 0.69±0.12 in SRP + SDD group, and 2.2±0.38 and 1.7±0.7 in SRP + Placebo group, at days 0 and 90 respectively. Mean differences of GI index values were detected as 1.55±0.16, 1.61±0.05 and 0.5±0.46 between days 0-90, in SRP + Probiotic, SRP + SDD and SRP + Placebo groups, respectively (Table 7-10). Inter-group comparisons of mean differences of GI values revealed statistically significant results in favor of SRP + Probiotic and SRP + SDD group compared to SRP + Placebo group between days 0-90 ($p=0.001$; $p= 0.001$, respectively), however no significant difference was detected between SRP + Probiotic and SRP + SDD group ($p=0.921$) (Table 7-10).

4.2.3. Probing Depth

No significant differences were detected between groups at baseline in PD values (Table 6). GI values were detected as 5.19±0.61 mm and 3.53±0.77 mm in SRP + Probiotic group, 5.59±0.21 mm and 3.9±0.21 mm in SRP + SDD group, and 5.13±1.05 mm, 4.61±1.08 mm in SRP + Placebo group, at days 0 and 90 respectively. Mean differences of PD values were detected as 1.66±0.22 mm, 1.7±0.17 mm and 0.52□0.16 mm between days 0-90, in SRP + Probiotic, SRP + SDD and SRP + Placebo groups, respectively (Table 7-10). Inter-group comparisons of mean differences of PD values

revealed statistically significant results in favor of SRP + Probiotic and SRP + SDD group compared to SRP + Placebo group between days 0-90 (p=0.001; p= 0.001, respectively), however no significant difference was detected between SRP + Probiotic and SRP + SDD group (p=0.944) (Table 7-10).

4.2.4. Attachment Gain

Negative changes in RAL values were determined as attachment gain. In the SRP + Probiotic group, mean attachment gain values were detected as 1.25±0.15 mm at day 90. In the SRP + SDD group mean attachment gain values were detected as 1.32±0.06 mm, at days 0-90. In the SRP + Placebo group mean attachment gain values were detected, 0.40±0.14 mm, at days 0-90 (Table 9). Inter-group comparisons of mean attachment gain values revealed statistical significant results in favor of the SRP + Probiotic and SRP + SDD groups compared to SRP + Placebo at day 90 (p =0.001; p=0.001) however no significant difference was detected between SRP + Probiotic and SRP + SDD group (p=0.787) (Table 9).

4.2.5. Bleeding on Probing

No significant differences were detected between groups at baseline in BoP values (Table 6). BoP values were detected as 86,5±13.5 and 15.67±4 in SRP + Probiotic group, 89.01±3.4 and 6.67±1.96 in SRP + SDD group, and 89.5±3.67 and 22.83±5.53 in SRP + Placebo group, at days 0 and 90 respectively. Mean differences of BoP values were detected as 70.83±11.5, 82.34±3.12 and 66.67±7.66 between days 0-90, in SRP + Probiotic, SRP + SDD and SRP + Placebo groups, respectively (Table 7-10). Inter-group comparisons of mean differences of BoP values revealed statistically significant results in favor of SRP + Probiotic and SRP + SDD group compared to SRP + Placebo group between days 0-90 (p=0.001; p= 0.001, respectively), however no significant difference was detected between SRP + Probiotic and SRP + SDD group (p=0.938) (Table 7-10).

Table 7. Intra-group and inter-group comparisons of the clinical parameters at day 0 and 90.

Clinical Parameters		**SRP+Probiotic Mean±SD**	**SRP+SDD Mean±SD**	**SRP+Placebo Mean±SD**	**[1]p**
PI	**0**	2.36±0.35	2.47±0.2	2.27±0.3	**0.507**
	90	0.55±0.19	0.62±0.16	1.25±0.18	**0.001**
	[2]p	**0.001**	**0.001**	**0.003**	
GI	**0**	2.15±0.17	2.3±0.11	2.2±0.38	**0.574**
	90	0.6±0.23	0.69±0.12	1.7±0.7	**0.001**
	[2]p	**0.001**	**0.001**	**0.043**	
BOP (%)	**0**	86.5±13.5	89.01±3.4	89.5±3.67	**0.801**
	90	15.67±4.27	6.67±1.96	22.83±5.53	**0.001**
	[2]p	**0.001**	**0.001**	**0.001**	
PD (mm)	**0**	5.19±0.61	5.59±0.21	5.13±1.05	**0.486**
	90	3.53±0.77	3.9±0.21	4.61±1.08	**0.080**
	[2]p	**0.001**	**0.001**	**0.001**	
RAL (mm)	**0**	10.27±0.87	10.16±1.07	10.03±0.85	
	90	9.02±0.85	8.84±1.03	9.63±0.87	
	[2]P	**0.001**	**0.001**	**0.001**	

[1]Oneway ANOVA [2]Paired samples t Test, $p< 0.05$

PI: Plaque index, GI: Gingival index, BoP: Bleeding on Probing, PD: Probing depth.
RAL: relative attachment level

Table 8: Inter-group comparisons of the mean differences of clinical parameters in pairs at day 0 and day 90.

Clinical Parameters		**p**	**SRP+Probiotic-SRP+SDD**	**SRP+Probiotic-SRP+Placebo**	**SRP+SDD-SRP+Placebo**
PI	**0**	**[1]p**	0.788	0.859	0.477
	90	**[1]p**	0.776	**0.001**	**0.001**
GI	**0**	**[1]p**	0.555	0.931	0.771
	90	**[1]p**	0.933	**0.001**	**0.003**
BOP (%)	**0**	**[1]p**	0.861	0.809	0.994
	90	**[1]p**	**0.005**	**0.025**	**0.001**
PD (mm)	**0**	**[1]p**	0.598	0.988	0.509
	90	**[1]p**	0.690	0.070	0.283

[1]Tukey HSD Test $p < 0.05$

Table 9: Inter-group comparisons of the mean difference in clinical parameters between days 0-90.

Clinical Parameters	**Group I. SRP +Probiotic (Mean±SD)**	**Group II. SRP + SDD (Mean±SD)**	**Group III. SRP + Placebo (Mean±SD)**	**[1]p**
PI	1.81±0.4	1.84±0.06	1.30±0.46	0.002
GI	1.55±0.16	1.61±0.05	0.5±0.46	0.001
BOP (%)	70.83±11.5	82.34±3.12	66.67±7.66	0.008
PD (mm)	1.66±0.22	1.7±0.17	0.52±0.16	0.001
Attachment gain (mm)	1.25±0.15	1.32±0.06	0.40±0.14	0.001

[1]*Oneway ANOVA p< 0.05*

Table 10: Inter-group comparisons of the mean differences of the clinical parameters in pairs at days 0-90.

Clinical Parameters		**SRP+Probiotic-SRP+SDD**	**SRP+Probiotic-SRP+Placebo**	**SRP+SDD-SRP+Placebo**
PI	[1]p	0.986	**0.004**	**0.003**
GI	[1]p	0.921	**0.001**	**0.001**
BOP (%)	[1]p	0.938	**0.001**	**0.001**
PD (mm)	[1]p	0.944	**0.001**	**0.001**
Attachment gain (mm)	[1]p	0.787	**0.001**	**0.001**

[1]*Tukey HSD Test p< 0.05*

4.3. Microbiological Data

The mean proportion values for obligate anaerobes and the values for TVC ($x10^5$ CFU/ml) (median-range) at baseline and days 90 for three groups are presented in (Table 11, 12). All three treatments led to a significant decrease of TVC ($x10^5$ CFU/ml) and proportions of obligate anaerobes at days 90 for all three groups ($p<0.005$) (Table 11, 12).

4.3.1. Total Viable Count and Proportions of Obligate Anaerobes

TVC values ($x10^5$ CFU/ml) (median-range) were found as 41 (35-43) and 11.2 (0.9-15) in the SRP + Probiotic group at days 0 and 90, respectively. In the SRP + SDD group TVC values were found as 33.5 (25-42) and 10 (0.5-12) at days 0 and 90,respectively, and in the SRP + Placebo group, TVC values were found as 42 (14-81) and 15.6 (10.2-60) at days 0 and 90, respectively. Intra-group comparisons of TVC values in all treatment groups revealed significant differences between day 0 and day 90 (0.005; 0.003; 0.005, respectively) (Table 11, 12). Mean differences of TVC values ($x10^5$ CFU/ml) (median-range) were detected as 29.8 (22-39.4) in SRP + Probiotic group, 25 (16-38.8) in SRP + SDD group and 17.4 (3.2-39.5) in the SRP + Placebo between days 0-90. Inter-group comparisons of mean differences of TVC ($x10^5$ CFU/ml) (median-range) values revealed statistical significant results in favor of the SRP + Probiotic group ($p=0.001$) at the end of day 90 (Table 11, 12).

The Proportions of obligate anaerobic bacteria values were found as 50.08±4.63 (48.02) and 19.31±4.51 (20) in the SRP + Probiotic group at day 0 and at day 90, in the SRP + SDD group it was detected as 48.60±4.49 (47.18) and 19.75±7.93 (19.75) at day 0 and 90, and in the SRP + Placebo group it was detected as 46.46±3.95 (44.81) and 33.26±5.76 (30.8) at day 0 and 90, respectively. Intra-group comparisons of proportions of obligate anaerobeic bacteria showed statistical significance between day 0 and day 90, in all three groups (0.005; 0.005; 0.003, respectively) (Table 11, 12). Inter-group comparisons of mean differences of the proportions obligate anaerobic bacteria values revealed statistical significant results in favor of the SRP + Probiotic and SRP + SDD groups. However no significant difference was detected between these groups ($p=0.149$).

Table 11: Intra-group and inter-group comparisons of microbiological parameters at baseline and day 90 and the mean differences between days 0-90.

TVC (x10^{5}CFU/ml)	**SRP+Probiotic Median (min-max)**	**SRP+SDD Median (min-max)**	**SRP+Placebo Median (min-max)**	**[1]p**
Day 0	41 (35-43)	33.5 (25-42)	42 (14-81)	0.086
Day 90	11.2 (0.9-15)	10 (0.5-12)	15.6 (10.2-60)	**0.004**
Day 0-90	29.8 (22-39.4)	25 (16-38.8)	17.4 (3.2-39.5)	**0.017**
[2]p	**0.005**	**0.003**	**0.005**	
Obligate Anaerobs (%)	**SRP+Probiotic Mean±SD**	**SRP+SDD Mean±SD**	**SRP+Placebo Mean±SD**	**[3]p**
Day 0	50.08±4.63	48.60±4.49	46.46±3.95	0.177
Day 90	19.31±4.51	19.75±7.93	33.26±5.76	**0.001**
Day 0-90	30.77±6.26	28.84±9.05	13.20±5.7	**0.001**
[4]p	**0.005**	**0.005**	**0.003**	

[1] Kruskal Wallis Test *[2]Wilcoxon sign test p<0.05* *[3] Oneway ANOVA test p<0.05*

[4]Paired Samples t Test

Table 12: Intergroup comparisons of microbiological parameters in pairs at day 0 and day 90 and intergroup comparisons of the mean differences in pairs between days 0-90.

TVC ($x10^5$ CFU/ml)		**SRP+Probiotic-SRP+SDD**	**SRP+Probiotic-SRP+ Placebo**	**SRP+SDD-SRP+Placebo**
Day 0	[1]p	0.728	0.156	0.508
Day 90	[1]p	0.986	0.001	0.001
Day 0-90	[1]p	0. 001	0.001	0. 818
Obligate Anaerobs (%)		**SRP+Probiotic-SRP+SDD**	**SRP+Probiotic-SRP+ Placebo**	**SRP+SDD-SRP+Placebo**
Day 0	[2]p	0.067	0.595	0.104
Day 90	[2]p	0.363	**0.020**	**0.003**
Day 0-90	[2]p	0.140	**0.001**	**0.001**

[1] *Mann Whitney U* [2] *Tukey HSD Test* $p < 0.05$

5.DISCUSSION

Periodontitis is defined as a chronic disease caused by multiple factors and components, including oral bacteria and the host immune system (129). Periodontal disease occurs in a susceptible host and in the presence of pathogenic species in combination with low concentrations of so-called ‘‘beneficial bacteria’’ (22). As it is the pathogenic bacteria present in the biofilm that initiate the disease, it is considered challengeable therapeutic areas (9).

SRP has become the ‘‘gold standard’’ of nonsurgical treatment of periodontitis. Multiple clinical studies demonstrated that it self effectively reduces the microbial load (139). However, some studies do not report significant microbial improvements after subgingival debridement, possibly because of insufficient oral hygiene follow-up and/or limited subgingival instrumentation. In the presence of poor oral hygiene, a pathogenic subgingival microflora may be already re-established within 8 to 12 weeks after a single debridement session (140, 141). In the last decades, different adjunctive therapeutic approaches have been proposed in order to long last the effectiveness of SRP and eventually lessen the need for periodontal surgery in advanced periodontitis patients. Three treatment approaches regarding SRP application have been proposed: (142, 143)

- Stage debridement with quadrant or sextant,
- Full-mouth SRP,
- Full-mouth disinfection. (144)

However, two systematic reviews stated that these three approaches are all effective and that the thoroughness of root debridement and the patient’s standard of oral hygiene are critical factors rather than the treatment modality (145).

Even though a shift in the composition of the oral microflora towards a less pathogenic species occurs after SRP, unfortunately complete elimination of periodontal pathogens is not possible (146). Although antiseptics and antibiotics have been used for many years, their therapeutic effectiveness has been found to be temporally and increase risk in the development of antibiotic resistance has been documented (147). Additionally, antibiotics target the bacteria and eliminate both pathogenic and beneficial species. On the other hand lasers and photodynamic therapy still need improvements in terms of their clinical and microbiological efficacy ın the periodontal treatment (10).

While bacteria are undoubtedly the principle cause of initial inflammatory lesions leading to gingivitis it is the host response and not the type of bacteria which dictates whether the disease will progress or not. In recent years greater emphasis has been placed on the host response and the change is to move away from a purely mechanistic view and consider the driving forces of the disease, namely uncontrolled inflammation in this emerging paradigm, it is suggested that if the inflammation can be controlled then so can the infection (17). So the HMT approach is the most promising treatment modality for the treatment of periodontal disease. Recently probiotics have been proposed as an adjunct to periodontal therapy in order to mediate in the host part of the disease.

SDD it is the only FDA approved HMT agent in the treatment of periodontitis (12). However the mechanism of action of probiotics its still unclear, but lately their suggested effects in the oral cavity can be broadly divided into three groups as follow (15):

- Modulation of the host inflammatory response,
- Direct effects against pathogenic bacteria,
- Indirect effects against pathogenic bacteria.

Since there is no study in the literature evaluating the effectiveness of both agents in comparison to each other, this study aimed to evaluate the effects of both agents as adjuncts to SRP on clinical and microbiological parameters in a 3-month follow-up period in CP patients.

The susceptibility of the host is partly hereditary (such as inadequate or unregulated immune response) but can be influenced by environmental and behavioral factors such as viral infections, smoking and stress (9). Smoking diminishes both cell-mediated and hummoral immune response on one hand and on the other hand it favors infection with microbial pathogens and impairs antimicrobial therapy. It was concluded that cigarette smoking appear to trigger a cycle of impaired immune responses and subgingival infection with periodontal pathogens leading to greater severity of periodontal disease (148). Therefore smokers were excluded from this study.

In the presence of a well-established microflora, exogenous pathogens experience difficulties in surviving and competing in the indigenous ecosystem leading to appreciate the commensal bacteria present in the oral flora. Since the commensal bacteria are essential in regulating the host defense and protecting against exogenous pathogens, disruption of the established flora by total removal of plaque by

mechanically is necessary before administration of any adjunctive agents (22). The sugbingival microflora is affected by supragingival quantitiy, composition and rate of accumulation. So the proper plaque removal is important for successful periodontal outcomes. Therefore, one week prior to the study period, every patient was instructed to brush their teeth by the modified Bass method and to use interdental brushes. Oral hygiene levels and accumulation of plaque deposits were evaluated by PI scores (147). Every patient was regularly checked for oral hygiene reinforcement at days 35 and 60. Studies have demonstrated that the major changes occur during the initial 1–3 months after completion of the nonsurgical periodontal treatment (155, 156) and data show that most of the healing occurs within 3 months. So in the present study, the evaluation period was determined as 3 months. Mean baseline PI scores in SRP + probiotic, SRP + SDD and SRP + placebo groups were detected as 1.81, 1.84 and 1.30 respectively. In all groups, PI scores showed statistically significant reduction at the end of the study period. This finding showed that all patients in the present study, provided optimal oral hygiene level throughout the study period.

GI and BoP scores were determined and gave an idea about the inflammatory status of the gingiva. In all three groups statistically significant reductions were observed throughout the study. In the presence of a well-established oral hygiene levels and in combination with SRP lead to a reduction in bleeding tendency and inflammation of the periodontium (155-159). Both GI and BoP scores were significantly reduced in all groups when compared to baseline values. Intergroup comparisons of mean differences of both parameters revealed significant results in favor of SRP + Probiotic and SRP+SDD groups. These results are in consistent with the other studies that reported reductions in GI and BoP scores after probiotic and SDD administration (55, 67, 71, 118, 123, 160, 161). Twetman et al. (123) evaluated the clinical and biochemical effect of a chewing gum containing probiotic on gingival inflammation in patients with gingivitis. The authors concluded that the pro-inflammatory cytokines such as IL-1, TNF, and IL-8 levels in GCF were significantly reduced by the consumption of probiotics. This may be the proof that probiotics do have a role in combating periodontal inflammation. Also, the usage of SDD as an adjunct to SRP had a great influence on GI and BoP scores. These results are similar to other studies that reported reductions in GI and BoP scores after SDD administration (55, 67, 71). Emingil et al. (55) reported that the SRP + SDD treatment showed a statistically significant improvement in GI score compared to the control group throughout the study period

(P<0.05). Gurkan et al. (67) reported that SRP plus SDD therapy resulted in statistically significant reduction in papilla bleeding index (PBI) when compared to SRP + Placebo group at 6 months (p<0.05). Additionally Gorska et al. (71) stated that the bleeding index (BI) was decreased in SRP+SDD and SRP groups after 3 months however the reduction in SRP+SDD group was reported higher and the difference between the groups was found to be statistically significant in favor of SRP+SDD group. There are also contradictory results are found in the literature that showed no clinical benefits of probiotic and SDD usage as adjuncts to SRP (122, 162). These studies were conducted either in healthy or experimental gingivitis patients without performing any mechanical periodontal therapy. In order for the agents to be effective, it is of importance to mechanically disrupt the mature biofilm. Therefore in the present study SRP was performed prior to SDD and probiotic administration.

Gain in clinical attachment level and recession of the marginal gingival tissues leads to the reduction in PD (158, 163). Gingival recession occurs due the reduction in the amount of swelling of the gingival margin. Inflammatory cell present in the tissue infiltrates, and more collagen-rich tissue replaces the increased numbers of capillaries present in the gingival connective tissue (164). Eventually shrinkage of the tissue in an apical direction and towards the root surface is observed. The interface between the root surface and the former pocket epithelium is partially transformed into a long-junctional epithelium (165, 166). The increase in the collagen fibers of the gingival connective tissue and the presence of the long-junctional epithelium, results in the gain of clinical attachment level, which leads to an increased resistance of the tissues against the penetration of a periodontal probe.

At the beginning of this study, individual acrylic stents with grooves were used as reference points and were prepared in order to standardize probe position and angulation in each measurement point. In all groups yielded significant results in PD reduction at the end day 90, however these reduction were found statistically significant in favor of the SRP + Probiotic and SRP+SDD groups. The amount of reduction was detected as 1.66 and 1.73 in SRP + Probiotic and SRP + SDD groups, respectively, Vivekananda et al. (131) reported a 1.31 mm PD reduction in the SRP + Probiotic group after 3 weeks of probiotics usage over the 42-day follow-up period. In another similar designed study conducted by Teughels et al. (132), revealed that the application of *L.reuteri* containing lozenges as an adjunct to SRP resulted in a faster PD reduction. For deep pockets, significantly lower mean PD reduction in the SRP + Probiotic group (1.41

mm) was observed when compared to the SRP + Placebo group, which was detected as 1.39 mm. For moderate and deep pockets, the SRP + Probiotic group showed a significantly larger PD reductions when compared to the SRP group, which was explained by "the deeper the pocket at baseline, the more pronounced the effect of the probiotic was". Furthermore in a study by SDD Emingil et al. (55) reported in a study of 12-month duration, that the reduction in PD was similar for both test and control groups at 3 and 6 months. However the test group showed a significantly higher reduction in PD than the placebo group at 9 and 12 months (P=0.0413, P=0.0233, respectively). Gorska et al. (71) reported that in patients treated with the SDD, the mean PD scores significantly decreased. After conventional treatment alone, the decrease in this parameter was not as marked, but the difference between the groups was significant. Also Gurkan et al. (67) reported no statistically significant changes at sites with a baseline PD 0–3 mm in both groups. However significant PD reductions were observed at sites with a baseline PD 4–6 mm and >7 mm and that this reduction was maintained over the entire study period ($p<0.025$). Additionally Emingil et al. (70) also showed a statistically significant decrease in PD values in the test group when compared to control group. This is accordance with the results of this present study. This may be attributed to the effect of the mechanical debridement with the strict recall visits scheduled and the significant reduction observed in PI and GI scores.

The amount of attachment gain levels obtained, should evaluated together with PD reduction. CAL or RAL measurements can be used in determining the changes in the attachment levels (179). However CAL is measured from a clinical landmark such as the cemento-enamel junction to the tip of the probe during probing, and in repeated measurements it may show to inherit errors and not to be reliable. Therefore in this study RAL were evaluated by using individual occlusal acrylic stents with grooves in order to minimize errors between different measurement intervals. Significant improvement of attachment gain for all three groups was observed. Attachment gain for SRP + Probiotic was 1.25 mm, SRP + SDD group was 1.32 mm and for SRP + Placebo group was 0,40 mm at day 90. Vivekananda et al. (132) detected an attachment gain of 1.09 mm in the SRP+ Probiotic group and 0.29 mm in the SRP+ Placebo group. Teughels et al. (22) reported an attachment gain of 1.42 mm in the test and 1.01 mm in the control group in moderate periodontal pockets. In deep pockets attachment gain was reported 1.47 mm for test and 0.67 mm for the control group. In the studies regarding the consumption of SDD Caton et al (75) revealed a mean change of 1.03mm of

attachment gain in the SRP + SDD group and 0.86mm in the control group in moderate periodontal pockets. In deep pockets attachment gain was reported as 1.55mm in the test group and 1.17mm in the control group. Preshaw et al (57) revealed a mean change of 1.27mm of attachment gain in the SRP + SDD group and 0.94mm in the control group in moderate periodontal pockets. In deep pockets attachment gain was reported as 2.09mm in the test group and 1.60mm in the control group. These findings are in accordance with the attachment gain observed in this study.

Microbiological parameters were evaluated by collecting subgingival plaque samples. Although collecting saliva samples may give consistent result in patients with periodontal inflammation, it is more relevant to study microbial composition of the gingival crevice (87). Therefore in this study design we obtained the microbiological samples from the GCF of each patient.

Non-cultural methods are mainly based on immune-diagnosis and nucleic acid-based detection method (167). Among them PCR provides a very specific and sensitive technique for an accurate detection of targeted microorganisms with species-specific and sensitive primers (168). However this method can detect both, viable and non-viable bacteria, therefore the diagnostic importance of PCR is immeasurable. Detecting non-viable microorganisms after antimicrobial therapy makes assessing the effectiveness of the antimicrobial agent difficult and unreliable (169). On the other hand culturing techniques have been the classic diagnostic method to detect bacterial species found in the subgingival microflora (99). Although, it has limitations such as difficulty in claiming cultivable species in low numbers, in addition strict requirements are needed, such as the need for experienced personnel, time and relatively high cost, it is still considered the gold standard in characterizing the subgingival microbiota (168).

Several pathogenic microorganisms have been found to colonize in deep pockets (PD ≥ 5mm) including red and orange complex bacteria (42, 170-172). Previous studies assessed the pathogenicity of periodontal disease from subgingival plaque samples obtained from periodontal sites with PD ≥ 5mm (173, 174). Therefore in this study all subgingival samples from CP patients were taken from single rooted teeth at sites with PD ≥ 5mm and GI ≥ 2.

A significant decreased in all groups were observed regarding TVC ($x10^5$ CFU/ml) and proportions of obligate anaerobes when compared to baseline values. Intergroup analysis of mean differences of TVC ($x10^5$ CFU/ml) values revealed significant results in favor of SRP + Probiotic group. It has been stated in previous

studies that, Lactobacilli exert an antimicrobial activity by producing antimicrobial substances (128). These bacteria have also been shown to activate immune-competent cells to secrete both inflammatory and anti-inflammatory cytokines, resulting in modulation of the mucosal immune system. Presumably, probiotics may exert their beneficial effect in the oral cavity by both direct interactions with microorganisms in dental plaque and indirect actions such as modulation of the innate/acquired immune systems. Therefore the significant decrease observed in SRP + Probiotic group regarding TVC values could be associated with this effect. On the other hand proportions of obligate anaerobes in SRP + SDD and SRP + Probiotic groups decreased significantly more than SRP + Placebo group ($p<0.05$). It may be speculated that SDD might exhibit an anti-inflammatory effect, which leads to lessen the nutrients, that supports the growth of pathogenic species (56) so that the significant microbial reduction that observed in SRP+SDD group compared to placebo-controlled group can be explained by this suggested mechanism. In a similar study by Tekce et al. (134) reported statistically significant difference between the groups with respect to the microbiological parameters in favour of the SRP + Probiotic group on days 21, 90 and 180 ($p < 0.05$). Although the identification of specific obligate anaerobic strains was not performed in this study, a statistically significant reduction in the percentage of obligate anaerobes was observed on days 21, 90 and 180 ($p < 0.05$). They reported that probiotics could be useful in the elimination of specific obligate anaerobes.

The most obvious changes in the total microbiota occur in the first 3 months, (138) which is consistent with our microbiological findings. Although identification of specific obligate anaerobic strains was not performed in this study, statistically significant reduction in percentage of obligate anaerobes was observed ($p<0.05$). This result is consistent with Vivekananda et al. (131), Iniesta et al. (124), Teughels et al. (132), Walker et al. (53), Novak et al. (56) Lee et al. (73) and Tekce et al (134). These studies demonstrated that probiotics and SDD were useful in the elimination of specific obligate anaerobes.

Limited data is available about the appropriate probiotic dosing regiments and only few dose-comparison studies have been undertaken (175). There are only few studies regarding the time of usage of probiotics in the treatment of CP. It would be too early to propose any clinical recommendations at this stage (133). In this study *L. reuteri* containing lozenges were prescribed twice a day for 3 months. However, FDA suggests SDD containing tablets to be used for three to nine months. In order to

standardize the duration of usage of the both agents, both of them were administered for 3 months in this present study.

The present randomized controlled clinical trial demonstrated the significant adjunctive effect of the usage of SDD containing tablets and probiotic containing lozenges in CP patients in a 3-month consumption in terms of clinical and microbiological parameters. Considering the clinical and microbiological outcomes of the SDD tablets and probiotic lozenges, these agents could be proposed as a beneficial adjunctive alternative in the non-surgical treatment of patients with CP. However, long-term clinical and microbiological studies in larger groups of patients are necessary in order to optimize the results.

6. References

1. Mahanonda R, Sa-Ard-Iam N, Rerkyen P, Champaiboon C, Vanavit N & Pichyangkul S. Innate antiviral immunity of periodontal tissue. *Periodontology 2000*. 2011; 56, 143–153.

2. Socransky SS, Haffajee AD. The bacterial etiology of destructive periodontal disease: current concepts. *J Periodontol*, 63:322–31, 1992.

3. Darveau RP, Tanner A, Page RC. The microbial challenge in periodontitis. *Periodontol 2000*. 1997; 14: 12-13.

4. Schaudinn C. Gorur A, Keller D, Sedghizadeh PP. Periodontitis: An Archetypical Biofilm Disease. *J Am Dent Assoc.* 2009; 140: 978-986.

5. Dommisch H, Kebschull M. Chronic periodontitis. In: Newman MG, Takei H, Klokkevold PR, Carranza FA (Eds). Carranza's Clinical Periodontology. (12th ed.) Elsevier Saunders, China. 313-318.

6. Rhemrev GE, Timmerman MF, Veldkamp I, Van Winkelhoff a J, Van der Velden U. Immediate effect of instrumentation on the subgingival microflora in deep inflamed pockets under strict plaque control. *J Clin Periodontol.* 2006; 33:42–8.

7. Sbordone L, Ramaglia L, Gulletta E, Iacono V. Recolonization of the subgingival microflora after scaling and root planing in human periodontitis. *J Periodontol*. 1990; 61: 579–84.

8. Claffey N, Polyzois I, Ziaka P. An overview of nonsurgical and surgical therapy. *Periodontol 2000*. 2004; 36: 35–44.

9. Quirynen M, Teughels W, De Soete M, Van Steenberghe D. Topical antiseptics and antibiotics in the initial therapy of chronic adult periodontitis: microbiological aspects. *Periodontol 2000*. 2002; 28: 72–90.

10. Yilmaz S, Kut B, Gursoy H, Eren-Kuru B, Noyan U, Kadir T. Er:YAG laser versus systemic metronidazole as an adjunct to nonsurgical periodontal therapy: a clinical and microbiological study. *Photomed Laser Surg.* 2012; 30(6): 325–30.

11. Yilmaz S, Algan S, Gursoy H Noyan U, Kuru BE, Kadir T. Evaluation of the clinical and antimicrobial effects of the Er:YAG laser or topical gaseous ozone as adjuncts to initial periodontal therapy. *Photomed Laser Surg*. 2013; 31(6): 293–8.

12. El-Shinnawi UM, El-Tantawy SI: The effect of alendronate sodium on alveolar bone loss in periodontitis (clinical trial). *J Int Acad Periodontol.* 2003; 5:5.

13. J. Caton. S. Ciancio, T. Blieden, M. Bradshaw, Richard J. Crout, Arthur F. heft, et al. Treatment with subantimicrobial dose doxycycline improves the efficacy of scaling and root planning in patients with adult periodontitis. *J Periodontol*. 2000; 71:521.

14. http://www.who.int/foodsafety/fs_management/en/probiotic_guidelines.pdf.

15. Laleman. I, Teughels. W Probiotics in the dental practice: A review. *Quintessence Int.* 2015; 46:255-264.

16. Taba M, Scombatti de Souza SL, Mariguela VC. Periodontal disease: a genetic perspective. *Braz. oral res*. 2012; 26.

17. P. Bartold, T. Van Dyke, Periodontitis: a host-mediated disruption of microbial homeostasis. Unlearning learned concepts. *Periodontology 2000*. 2013; 62: 203–217.

18. Page RC, Kornman K. The pathogenesis of human periodontitis: an introduction. *Periodontol 2000.* 1997; 14: 10– 19.

19. Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent RL Jr. Microbial complexes in subgingival plaque. Antimicrobial effects of mechanical debridment. *J Clin Periodontol*. 1998; 25: 134–144.

20. Socransky SS, Haffajee AD. Dental biofilms: difficult therapeutic targets. *Periodontol 2000.* 2002; 28: 12–55.

21. Sanz M, Winkelhoff A.J.V. Periodontal infections: understanding the complexity-consensus of the seventh europeon workshop on periodontology. *J clin periodontol.* 2011; 38.

22. Teughels W, Essche M Van, Sliepen I, Quirynen M. Probiotics and oral healthcare. *Periodontol 2000*. 2008; 48:111–47.

23. Marsh PD, Moter A, Devine DA. Dental plaque biofilms: communities, conflict and control. *Periodontol 2000*. 2011; 55: 16–35.

24. Flemming T. Periodontitis. *Ann Periodontol*. 1999; 4:32.

25. Novak M, Novak K. Chronic Periodontitis. In: Newman MG, Takei H, Klokkevold PR, Carranza FA, editors. Carranza's Clinical Periodontology. 11th ed. China: Elsevier Saunders; p. 160–8.

26. Armitage GC. Development of a classification system for periodontal diseases and conditions. *Ann Periodontol*. 1999; 4:1–6.

27. Page RC, Kornman KS. The pathogenesis of human periodontitis: an introduction. *Periodontol 2000*. 1997; 14: 9-11.

28. Petersilka GJ, Ehmke B, Flemmig TF. Antimicrobial effects of mechanical debridement. *Periodontol 2000*. 2002; 28(166): 56–71.

29. Carranza F, Takei H. The Treatment Plan. In: Newman M., Takei H, Klokkevold P, Carranza F, editors. Carranza's Clinical Periodontology. 10th ed. Los Angelis, California: Saunders. 2007; 626–629.

30. Maiden MF, Tanner A, McArdle S, Najpauer K, Goodson JM. Tetracycline fiber therapy monitored by DNA probe and cultural methods. *J Peridont Res*. 1991; 26:452–9.

31. Cionca, N, Giannopoulou C, Ugolotti G & Mombelli. Amoxicillin and metronidazole as an adjunct to full-mouth scaling and root planning of chronic periodontitis. *J periodont.* 2009; 80: 364–371.

32. Magnusson I, Lindhe J, Yoneyama T, Liljenberg B. Recolonization of a subgingival microbiota following scaling in deep pockets. *J Clin Periodontol*. 1984; 11:193–207.

33. Haffajee D, Cugini M , Dibart S, Smith C, Kent RL. The Effect Of SRP On The Clinical And Microbiological Parameters Of Periodontal Diseases. *J Clin Periodontol*. 1997; 24(5): 324–34.

34. Harper DS, Robinson PJ. Correlation of histometric, microbial, and clinical indicators of periodontal disease status before and after root planing. *J Clin Periodontol*. 1987; 14:190–196.

35. Buchannan SA, Robertson PJ. Calculus removal by scaling/ root planing with and without surgical access. *J Periodontol*. 1987; 58: 159–163.

36. Stambaugh R, Dragoo M, Smith DM, Carasali L. The limits of subgingival scaling. *Int J Periodontics Restorative Dent.* 1981; 1: 30–41.

37. Dragoo MR. A clinical evaluation of hand and ultrasonic instruments on subgingival debridement. 1. With unmodified and modified ultrasonic inserts. *Int. J Periodontics Restorative Dent.* 1992; 12: 311–323.

38. Danser MM, van Winkelhoff AJ, de Graaff J, Loos BG, van der Velden U. Short-term effect of full-mouth extraction on periodontal pathogens colonizing the oral mucous membranes. *J Clin Periodontol.* 1994; 21: 484–489.

39. Quirynen M, De Soete M, Dierickx K, van Steenberghe D. The intra-oral translocation of periodontopathogens jeopardises outcome of periodontal therapy. A review of the literature. *J Clin Periodontol.* 2001; 28: 499–507.

40. Adriaens PA, De Boever JA, Loesche WJ. Bacterial invasion in root cementum and radicular dentin of periodontally diseased teeth in humans. A reservoir of periodontopathic bacteria. *J Periodontol.* 1988; 59: 222–230.

41. Renvert S, Dahle´n G, Wikström M. The clinical and microbiological effects of non-surgical periodontal therapy in smokers and non-smokers. *J Clin Periodontol.* 1998; 25: 153–157.

42. Berezow AB, Darveau RP. Microbial shift and periodontitis. *Periodontol 2000*. 2011; 55:36–47.

43. Teughels W, Newman MG, Coucke W, Haffajee AD, Van Der Mei HC, Kinder Haake S, et al. Guiding Periodontal Pocket Recolonization&: a Proof of Concept. *J Dent Res*. 2007; 86:1078–82.

44. Socransky SS, Haffajee A. Dental biofilms: difficult therapeutic targets. *Periodontol 2000*. 2002; 28:12–55.

45. Williams RC: Periodontal disease. *N Engl J Med.* 1990; 322 (6): 373.

46. Golub LM, Suomalainen K, Sorsa T: Host modulation with tetracyclines and their chemically modified analogues. *Curr Opin Dent.* 1992; 2:80.

47. Guggenberger. R, Koral. E, Zemann. W, Jacobsen C, Andreisek G, Metzler P: Cone beam computed tomography for diagnosis of bisphosphonate-related osteonecrosis of the jaw: evaluation of quantitative and qualitative image parameters. *Skeletal Radiol*. 2014; 43:1669–1678.

48. Rocha M, Nava LE, Vazquez de la Torre C, et al. Clinical and radiological improvement of periodontal disease in patients with type 2 diabetes mellitus treated with alendronate: a randomized, placebo-controlled trial. *J Periodontol.* 2001; 72:204.

49. Carter G, Goss AN, Doecke C: Bisphosphonates and avascular necrosis of the jaw: a possible association. *Med J Aust.* 2005; 182 (8):413.

50. Stephen G. Walker, Lorne M. Golub. Host Modulation Therapy for Periodontal Disease: Subantimicrobial-dose doxycycline, Medical as well as Dental Benefits. 2012.

51. Golub LM, Wolff M, Lee HM. Further evidence that tetracyclines inhibit collagenase activity in human crevicular fluid and from other mammalian sources. *J Periodont Res.*1985; 20:12.

52. Thomas J, Walker C, Bradshaw M: Long-term use of subantimicrobial dose doxycycline does not lead to changes in antimicrobial susceptibility. *J Periodontol.* 2000; 71:1472.

53. Walker C, Thomas J, Nango S, et al. Long-term treatment with sub-antimicrobial dose doxycycline exerts no antibacterial effect on the subgingival microflora associated with adult periodontitis. *J Periodontol.* 2000; 71:1465.

54. Walker C, Preshaw PM, Novak J, et al. Long-term treatment with subantimicrobial dose doxycycline has no antibacterial effect on intestinal flora. *J Clin Periodontol.* 2005; 32:1163.

55. Emingil G, Atilla G, Sorsa T, et al. The effect of adjunctive low-dose doxycycline therapy on clinical parameters and gingival crevicular fluid matrix metalloproteinase-8 levels in chronic periodontitis. *J Periodontol.* 2004; 75:106.

56. Novak MJ, Johns LP, Miller RC, et al. Adjunctive benefits of subantimicrobial dose doxycycline in the management of severe, generalized, chronic periodontitis. *J Periodontol.* 2002; 73:762.

57. Preshaw PM, Hefti AF, Novak MJ, et al. Subantimicrobial dose doxycycline enhances the efficacy of scaling and root planing in chronic periodontitis: a multi-center trial. *J Periodontol.* 2004; 75:1068.

58. Thomas JG, Metheny RJ, Karakiozis JM, et al. Long-term sub-antimicrobial doxycycline (Periostat) as adjunctive management in adult periodontitis: effects on subgingival bacterial population dynamics. *Adv Dent Res.* 1998; 12:32.

59. Walker C, Puumala S, Golub LM, et al. Subantimicrobial dose doxycycline effects on osteopenic bone loss: microbiologic results. *J Periodontol.* 2007; 78 (8):1590.

60. Monk E, Shalita A and Siegel DM. Clinical applications of non-antimicrobial tetracyclines in dermatology. *Pharmacol. Res*. 2011; 63: 130-145.

61. Cohen DM, Lee HM, Bhattacharyya I et al. Effective treatment of cicatricial pemphigoid using a novel combination therapy. *J. Dent. Res*. 2000; 79:627.

62. Greenwald RA. The road forward: the scientific basis for tetracycline treatment of arthritic disorders. *Pharmacol. Res*. 2011; 64: 610-613.

63. Moses MA, Harper J and Folkman J. Doxycycline treatment for lymphangioleiomyomatosis with urinary monitoring for MMPs. *N. Engl. J. Med*. 2006; 354: 2621-2622.

64. Grossi SG, Skupcinski FB, DeCaro T. et al. Treatment of Periodontal disease in diabetics reduced glycated hemoglobin. *J. Periodontol*. 1997; 68: 713-719.36.

65. Payne JB, LM Golub. Using tetracyclines to treat osteoporotic/osteopenic bone loss: From the basic science laboratory to the clinic. *Pharma Res*. 2011; 63 (2), 121-129.

66. Blankenberg S, Rupprecht HJ, Poirier O. Plasma concentrations and genetic variation of matrix metalloproteinase 9 and prognosis of patients with cardiovascular disease. Circulation. 2003; 107 (12): 1579-1585.

67. Gürkan A, Cınarcık S, Hüseyinov A. Adjunctive subantimicrobial dose doxycycline: effect on clinical parameters and gingival crevicular fluid transforming growth factorβ1 levels in severe, generalized chronic periodontitis. *J Clin Peridontol*. 2005; 32: 244–253.

68. Emingil G, Atilla G, Sorsa T, Savolainen P, Baylas H. Effectiveness of adjunctive low-dose doxycycline therapy on clinical parameters and gingival crevicular fluid laminin-5 γ2 chain levels in chronic periodontitis. *J Periodontol*. 2004; 75:1387-1396.

69. Emingil G, Gürkan A, Atilla G, Kantarci A, Subantimicrobial-dose doxycycline and cytokine-chemokine levels in gingival crevicular fluid, *J Periodontol*. 2011; 82:452-461.

70. Emingil G, Gürkan A, Atilla G, Berdeli A, Çınarcık S. Adjunctive low-dose doxycycline therapy effect on clinical parameters and gingival crevicular fluid tissue plasminogen activator levels in chronic periodontitis, *Inflamm. Res*. 2006; 55; 550–558.

71. R. Górska, M. Góra. The effects of the initial treatment phase and of adjunctive low-dose doxycycline therapy on clinical parameters and MMP-8, MMP-9, and TIMP-1 levels in the saliva and peripheral blood of patients with chronic periodontitis. *Arch. Immunol. Ther. Exp*. 2006; 54; 419–426.

72. Needleman I, Suvan J, Gilthorpe MS, Tucker R, St George G, Giannobile W, et al. A randomized-controlled trial of low-dose doxycycline for periodontitis in smokers. *J Clin Periodontol*. 2007; 34: 325–333.

73. Lee JY, Lee YM, Shin SY, Seol YJ. Effect of subantimicrobial dose doxycyline as an effective adjunct to scaling and root planing, *J Periodontol*. 2004; 75; 1500-1508.

74. Gapski R, Barr JL, Sarment DP, et al. Effect of systemic matrix metalloproteinase inhibition on periodontal wound repair: a proof of concept trial. *J Periodontol.* 2004; 75:441.

75. Caton J, Ryan ME. Clinical studies on the management of periodontal disease utilizing subantimicrobial dose doxycycline (SDD). *Pharmacol. Rec*. 2011; 63: 114-120.

76. Metchnikoff E. The prolongation of life: Optimistic studies. London: William Heinemann. *Int J Food Microbio*. 1998; 39:237-238.

77. Meurman JH, Stamatova I. Probiotics: contributions to oral health. *Oral Dis*. 2007; 13: 443–451.

78. Kollath W. Nutrition and the tooth system general review with special reference to vitamins. *Dtsch Zahnarztl Z*. 1953; 8:l 7-16.

79. Lilly D, Stillwell R. Probiotics: growth-promotig factors produced by microorganisms. Science. 1965; 147:747–748.

80. Parker R. Probiotics, the other half of the antibiotic story. *Anim Nutr Heal*. 1974; 29:4–8.

81. Fuller R. Probiotics in man and animals. *J Appl Bacteriol*. 1989; 66:365–78.

82. Havennaar R, Huis In't Veld M. Probiotics: a general view. In: Wood B, editor. The Lactic Acid Bacteria. 8th ed. New York, USA: Springer; 1992; 151–70.

83. Schaafsma G. State of the art concerning probiotic strains in milk products. *IDF Nutr News*. 1996; 5:23–4.

84. Naidu A, Bidlack W, Clemens R. Probiotic spectra of lactic acid bacteria (LAB). *Crit Rev Food Sci Nutr*. 1999; 39:13–126.

85. Salminen S, Ouwehand A, Benno Y, Lee YK. Probiotics: how should they be defined? *Trends Food Sci Techno.* 1999; 10:8–11.

86. Schrezenmeir J, de Vrese M. Probiotics, prebiotics, and synbiotics: approaching a definition. *AM J Clin Nutr*. 2001; 73:361–364.

87. Stamatova I, Meurman JH. Probiotics And Periodontal Disease. *Periodontol 2000*. 2009; 51: 141–151.

88. Gupta V, Garg R. Probiotics. *Indian J Med Microbiol*. 2009; 27:202–9.

89. Caglar E, Kargul B, Tanboga I. Bacteriotherapy and probiotics' role on oral health. *Oral Dis*. 2005; 11(3):131–7.

90. Singh VP, Sharma J, Babu S, Rizwanulla S . A Role of probiotics in health and disease: A review *J Pak Med Assoc.* 2013; 63: 2.

91. Stamatova I, Jukka H. Meurman. Probiotics: Health benefits in the mouth. *American J Dent*. 2009; 22: 6.

92. Mauli Simratvir Kaur, Parampreet K. PannuVirat Galhotra. Probiotics: A new way to maintain oral health. *Ind J Dent.* 2012; 3: 2.

93. Sinkiewicz G, Cronholm S, Ljunggren L, Dahlén G, Bratthall G. Influence of dietary supplementation with Lactobacillus reuteri on the oral flora of healthy subjects. *Swed Dent J*. 34:197-206, 2010.

94. Axelsson L. Lactic acid bacteria: Classification and Physiology. In: Salminen S, von Wright A, Ouwehand A, editors. Lactic acid bacteria: microbiology and functional aspects. Third Edit. New York, *Basel: Marcek Dekker*. 2004; 1–72.

95. Reuter G. The Lactobacillus and Bifidobacterium microflora of the human intestine: composition and succession. *Curr Issues Intest Microbiol.* 2001; 2(2):43–53.

96. Kandler O, Stetter K, Köhl R. Lactobacillus reuteri sp. a new species of heterofermentative lactobacilli. *Zbl Bakt Hyg*. 1980; 1:264–9.

97. Felis GE, Dellaglio F. Taxonomy of Lactobacilli and Bifidobacteria. *Curr Issues Intest Microbiol*. 2007; 8:44–61.

98. El-Ziney MG, Debevere JM. The effect of Reuterin on Listeria monocytogenes and Escherichia coli O157:H7 in milk and cottage cheese. *J Food Prot*. 1998; 61:1275– 80.

99. Savino F, Cordisco L, Tarasco V, Palumeri E, Calabrese R, Oggero R, et al. Lactobacillus reuteri DSM 17938 in infantile colic: a randomized, double-blind, placebo-controlled trial. *Pediatrics*. 2010; 126:526–533.

100. Valeur N, Engel P, Carbajal N, Connolly E, Ladefoged K. Colonization and immunomodulation by Lactobacillus reuteri ATCC 55730 in the human gastrointestinal tract. *Appl Environ Microbiol*. 2004; 70(2):1176–81.

101. Weizman Z, Asli G, Alsheikh A. Effect of a probiotic infant formula on infections in child care centers: comparison of two probiotic agents. *Pediatrics*. 2005; 115(1):5–9.

102. Talarico TL, Casas IA, Chung TC, Dobrogosz WJ. Production and isolation of reuterin, a growth inhibitor produced by Lactobacillus reuteri. *Antimicrob Agents Chemother*. 1988; 32:1854–8.

103. Gänzle MG, Höltzel A, Walter J, Jung G, Hammes WP. Characterization of reutericyclin produced by Lactobacillus reuteri LTH2584. *Appl Environ Microbiol*. 2000; 66:4325–33.

104. Nikawa H, Makihira S, Fukushima H, Nishimura H, Ozaki Y, Ishida K, et al. Lactobacillus reuteri in bovine milk fermented decreases the oral carriage of mutans streptococci. *Int J Food Microbiol*. 2004; 95:219–23.

105. Casas IA, Dobrogosz WJ. Validation of the Probiotic Concept&: Lactobacillus reuteri Confers Broad-spectrum Protection against Disease in Humans and Animals. *Microb Ecol Health Dis*. 2000; 10:247–85.

106. Chung TC, Axelsson L, Lindgren SE, Dobrogosz WJ. In Vitro Studies on Reuterin Synthesis by Lactobacillus. *Microb Ecol Health Dis*. 1989; 2:137–44.

107. Teughels W, Loozen G, Quirynen M: Do probiotics offer opportunities to manipulate the periodontal oral microbiota? *J Clin Periodontol* . 2011; 38: 159–177.

108. Mann GV, Spoery A. Studies of a surfactant and cholesteremia in the maassai. *Am J Clin Nut.* 1974; 23: 4649.

109. Hunter JO, Lee AJ, King TS. Enterococcus faecium strain PR88 - an effective probiotic. *Gut.* 1996; 38: A62.

110. Wilson M. Microbial inhabitants of humans: their ecology and role in oral health and disease. Cambridge University Press, Uk; 2005.

111. Devine D, Cosseau C. Host defense peptides in the oral cavity. Advances in Applied Microbiology. 2008; 281–322.

112. Wade W. Unculturable bacteria--the uncharacterized organisms that cause oral infections. *J R Soc Med*. 2002; 95:81–83.

113. Paster BJ, Olsen I, Aas J a, Dewhirst FE. The breadth of bacterial diversity in the human periodontal pocket and other oral sites. *Periodontol 2000*. 2006; 42:80–87.

114. Marsh PD. Dental plaque: biological significance of a biofilm and community life-style. *J Clin Periodontol*. 2005; 32:7–15.

115. Roberts A, Mullany P. Genetic basis of horizontal gene transfer among oral bacteria. *Periodontol 2000*. 2006; 42:36–46.

116. Blum S, Haller D, Peifer A, Schiffrin E. Probiotics and immune response. *Clin Rev Allergy Immunol*. 2002; 22:287–309.

117. Haukioja A, Yli-Knuuttila H, Loimaranta V, Kari K, Ouwehand AC, Meurman JH, et al. Oral adhesion and survival of probiotic and other lactobacilli and bifidobacteria in vitro. *Oral Microbiol Immunol*. 2006; 21:326–332.

118. Krasse P, Carlsson B, Dahl C. Decreased gum bleeding and reduced gingivitis by the probiotic Lactobacillus reuteri. *Swed Dent J*. 2005; 30(2): 55–60.

119. Iwamoto T, Suzuki N, Tanabe K, Takeshita T, Hirofuji T. Effects of probiotic Lactobacillus salivarius WB21 on halitosis and oral health: an open-label pilot trial. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2010; 110:201– 208.

120. Hatakka K, Ahola A, Yli-Knuuttila H, Richardson M, Poussa T, Meurman JH, et al. Probiotics reduce the prevelance of oral Candida in the elderly- a randomized contolled trial. *J Dent Res*. 2007;86:125–130.

121. Serino G, Rosling B, Ramberg P, Hellstrom MK, Socransky SS, Lindhe J. The effect of systemic antibiotics in the treatment of patients with recurrent periodontitis. *J Clin Periodontol*. 2001; 28: 411-418.

122. Staab B, Eick S, Knöfler G, Jenntsch H. The influence of a probiotic milk drink on the development of gingivitis&: a pilot study. *J Clin Periodontol*. 2009; 36:850–856.

123. Twetman S, Derawi B, Keller M, Ekstrand KIM, Yucel-lindberg LAY, Steckse C. Short-term effect of chewing gums containing probiotic Lactobacillus reuteri on the levels of inflammatory mediators in gingival crevicular fluid. *Acta Odontol Scand*. 2009; 67:19–24.

124. Iniesta M, Herrera D, Montero E, Zurbriggen M, Ar M, Mj M, et al. Probiotic effects of orally administered Lactobacillus reuteri -containing tablets on the subgingival and salivary microbiota in patients with gingivitis .A randomized clinical trial. *J Clin Periodontol*. 2012; 39:736–44.

125. Koll-Klais P, Mandar R, Leibur E, Marcotte H, Hammarstrom L. Oral lactobacilli in chronic periodontitis and periodontal health&: species composition and antimicrobial activity. *Oral Microbiol Immunol*. 2005; 20:354–361.

126. Ishikawa H, Aiba Y, Nakanishi M, Oh-hashi Y , Koga Y. Suppression of Periodontal Pathogenic Bacteria in the Saliva of Humans by the Administration of Lactobacillus salivarius TI 2711. *J Japanese Soc Periodontol*. 2003; 45:105–112.

127. Shimauchi H, Mayanagi G, Nakaya S, Minamibuchi M, Ito Y, Yamaki K, et al. Improvement of periodontal condition by probiotics with Lactobacillus salivarius WB21: a randomized, double-blind, placebo-controlled study. *J Clin Periodontol*. 2008; 35:897–905.

128. Mayanagi G, Kimura M, Nakaya S, , Hirata H, Sakamoto M, Benno Y, et al. Probiotic effects of orally administered Lactobacillus salivarius WB21- containing tablets on periodontopathic bacteria&: a controlled, randomized clinical trial. *J Clin Periodontol*. 2009; 36:506–13.

129. Tsubura S, Mizunuma H, Ishikawa S, Oyake I, Okabayashi I, Katoh K, et al. The effect of Bacillus subtilis mouth rinsing in patients with periodontitis. *J Clin Microbiol Infect Dis*. 2009; 28:1353–1356.

130. Laleman I, Yilmaz E, Ozcelik O, Haytac C, Pauwels M, Herrero ER, et al. The effect of a streptococci-containing probiotic in periodontal therapy: a randomized controlled trial. *J Clin Periodontol*. 2015 [E pub ahead of print]

131. Vivekananda MR, Vandana KL, Bhat KG. Effect of the probiotic Lactobacilli reuteri (Prodentis) in the management of periodontal disease: a preliminary randomized clinical trial. *J Oral Microbiol*. 2010; 2:1–9.

132. Teughels W, Durukan A, Ozcelik O, Pauwels M, Quirynen M, Haytac Mehmet C. Clinical and microbiological effects of Lactobacillus reuteri probiotics in the treatment of chronic periodontitis: a randomized placebo-controlled study. *J Clin Periodontol*. 2013; 40:1025–35.

133. Vicario M, Santos A, Violant D, Nart J, Giner L. Clinical changes in periodontal subjects with the probiotic Lactobacillus reuteri Prodentis: a preliminary randomized clinical trial. *Acta Odontol Scand*. 2013; 71:813–9.

134. Tekce M, Ince G, Gursoy H, Dirikan Ipci S, Cakar G, Kadir T, et al. Clinical and microbiological effects of probiotic lozenges in the treatment of chronic periodontitis: a 1-year follow-up study. *J Clin Periodontol.* 2015; 42(4):363-72.

135. Ince G, Gürsoy H, İpçi ŞD, Cakar G, Emekli-Alturfan E, Yılmaz S. Clinical and Biochemical Evaluation of Lozenges Containing Lactobacillus reuteri as an Adjunct to Non-Surgical Periodontal Therapy in Chronic Periodontitis. *J Periodontol*. 2015; 86(6): 746-54.

136. Silness J, Löe H. Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal conditioning. *Acta Odont Scand*. 1964; 22: 121-135.

137. Löe H, Silness J. Periodontal disease in pregnancy. I. Prevalence and severity. *Acta Odont Scand*. 1963; 21: 533-51.

138. Schwarz F, Sculean A, Berakdar M, Georg T, Reich E, Becker J. Clinical evaluation of an Er:YAG laser combined with scaling and root planing for non- surgical periodontal treatment. A controlled, prospective clinical study. *J Clin Periodontol*. 2003; 30:26–34.

139. Sanz I, Alonso B, Carasol M, Herrera D, Sanz M. Nonsurgical treatment of periodontitis. *J Evid Based Dent Pract*. Elsevier Inc. 2012; 12:76–86.

140. Mousque`s T, Listgarten MA, Phillips RW. Effect of scaling and root planing on the composition of the human subgingival microbial flora. *J Periodontal Res.* 1980; 15: 144–151.

141. Sbordone L, Ramaglia L, Gulletta E, Iacono V. Recolonization of the subgingival microflora after scaling and root planing in human periodontitis. *J Periodontol.* 1990; 61: 579–584.

142. Lang N, Tan W, Krahenmann M, Zwahlen M. A systematic review of the effects of full-mouth debridement with and without antiseptics in patients with chronic periodontitis. *J Clin Periodontol*. 2008; 35:8–21.

143. Eberhard J, Jervoe-Storm P, Needleman I Worthington H, Jepsen S. Full-mouth treatment concepts for chronic periodontitis: a systematic review. *J Clin Periodontol*. 2008; 35:591–604.

144. Quirynen M, Bollen CM, Vandekerckhove BN, Dekeyser C, Papaioannou W, Eyssen H. Full- versus partial-mouth disinfection in the treatment of periodontal infections: short-term clinical and microbiological observations. *J Dent Res*. 1995; 74: 1459– 1467.

145. Heitz-Mayfield LJ, Lang NP. Surgical and nonsurgical periodontal therapy. Learned and unlearned concepts. *Periodontol 2000*. 2013; 62:218–231.

146. Roberts FA, Darveau RP. Beneficial bacteria of the periodontium. *Periodontol 2000*. 2002; 30:40–50.

147. Loozen G, Cerci B, Essche M Van, Quirynen M, Teughels W. Oral Flora and Oral Care with Probiotics. *Int J Probiotics Prebiotics*. 2010; 5:1–18.

148. Zambon JJ, Grossi SG, Machtei EE, Ho a W, Dunford R, Genco RJ. Cigarette smoking increases the risk for subgingival infection with periodontal pathogens. *J Periodontol*. 1996; 67:1050–1054.

149. Page RC, Kornman KS. The pathogenesis of human periodontitis: An introduction. *Periodontol 2000*. 1997; 14:9.

150. Page RC. Milestones in periodontal research and the remaining critical issues. *J Periodontal Res*. 1999; 34:331.

151. Elavarasu S, Seker S, Murugan T. Host modulation by therapeutic agents. *J Pharm Bioallied Sci*. 2012; S256–S259.

152. Ingman T, Sorsa T, Konttinen YT. Salivary collagenase, elastase and tyripsin- like proteases as biochemical markers of periodontal tissue destruction in adult and localized juvenile periodontitis. *Oral Microbiol Immunol*. 1993; 8: 298-305.

153. Haukioja A, Loimaranta V, Tenovuo J. Probiotic bacteria affect the composition of salivary pellicle and Streptococcal adhesion in vitro. *Oral Microbiol Immunol*. 2008; 23: 336– 343.

154. Caton JG, Zander HA. The attachment between tooth and gingival tissues after periodic root planning and soft tissue curettage. *J Periodontol*. 1979; 50: 462- 466.

155. Badersten A, Nilveus R, Egelberg J. Effect of nonsurgical therapy. II. Severely advanced periodontitis. *J Clin Periodontol*. 1984; 11:63–76.

156. Cugini M, Haffajee A, Smith C, Kent R. The effect of scaling and root planing on the clinical and microbiological parameters of periodontal diseases. 12- month results. *J Clin Periodontol*. 2000; 27:30–36.

157. Ryan ME. Nonsurgical approaches for the treatment of periodontal diseases. *Dent Clin North Am*. 2005; 49:611–636.

158. Proye M, Caton J, Polson A. Initial healing of periodontal pockets after a single episode of root planning monitored by controlled probing forces. *J Periodontol*. 1982; 53:296–301.

159. Greenstein G. Periodontal response to mechanical nonsurgical therapy: a review. *J Periodontol*. 1992; 63:118–130.

160. Riccia DN, Bizzini F, Perilli MG, Polimeni A, Trinchieri V, Amicosante G, et al. Anti-inflammatory effects of Lactobacillus brevis (CD2) on periodontal disease. *Oral Dis*. 2007; 13:376–85.

161. Kang MS, Kim BG, Chung J, Lee HC, Oh JS. Inhibitory effect of Weissella cibaria isolates on the production of volatile sulphur compounds. *J Clin Periodontol*. 2006; 33:226–232.

162. Hallström H, Lindgren S, Yucel-Lindberg T, Dahlén G, Renvert S, Twetman S. Effect of probiotic lozenges on inflammatory reactions and oral biofilm during experimental gingivitis. *Acta Odontol Scand*. 2013; 71:828–833.

163. Hughes R, Caffesse R. Gingival changes following scaling and root planing and oral hygiene. A biometric evaluation. *J Periodontol*. 1987; 49:245–252.

164. Adriaens P, Adriaens L. Effects of nonsurgical periodontal therapy on hard and soft tissues. *Periodontol 2000*. 2004; 36:121–145.

165. Caton J, Zander H. The attachment between tooth and gingival tissues after periodic root planning and soft tissue curettage. *J Periodontol*. 1979; 50:462–466.

166. Caton J, Nyman S, Zander H. Histometric evaluation of periodontal surgery. Part II. Connective tissue attachment levels after four regenerative procedures. *J Clin Periodontol*. 1980; 7:224–231.

167. Chen C, Slots J. Microbiological tests for Actinobacillus actinomycetemcomitans and Porphyromonas gingivalis. *Periodontol 2000*. 1999; 20:53–64.

168. Lau L, Sanz M, Herrera D, Morillo JM, Martín C, Silva A. Quantitative real-time polymerase chain reaction versus culture: a comparison between two methods for the detection and quantification of Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis and Tannerella forsythensis in subgingival plaque samples. *J Clin Periodontol*. 2004; 31:1061–1069.

169. Kshitish D, Laxman VK. The use of ozonated water and 0.2% chlorhexidine in the treatment of periodontitis patients: a clinical and microbiologic study. *Indian J Dent Res*. 2010; 21:341–348.

170. Katsanoulas T, Renee I, Attsröm R. The effect of supragingival plaque control on the composition of the subgingival flora in periodontal pockets. *J Clin Periodontol*. 1992; 19:760–765.

171. American Academy of Periodontology. The Pathogenesis of Periodontal Diseases. *J Periodontol*. 1999; 457–470.

172. Kinane DF, Attström R. Advances in the pathogenesis of periodontitis. Group B consensus report of the fifth European Workshop in Periodontology. *J Clin Periodontol*. 2005; 32:130–131.

173. Ando Y, Aoki A, Watanabe H, Ishikawa I. Bactericidal effect of Erbium YAG laser on periodontopathic bacteria. *Laser Surg Med*. 1996; 19:190–200.

174. Daly C, Mitchel D, Highfield J, Grossberf D, Stewart D. Bacteremia due to periodontal probing: A clinical and microbiological investigation. *J Periodontol*. 2001; 72: 210–214.

175. Saxelin M, Elo S, Salminen S, Vapaatalo H. Dose response colonization of faeces after oral administration of Lactobacillus casei strain GG. *Microb Ecol Health Dis*. 1991;4: 209–214.

APPENDIX **1:**

YEDİTEPE ÜNİVERSİTESİ HASTANESİ	**YEDI□TEPE ÜNI□VERSI□TESI□ TIP FAKÜLTESI□ KLI□NI□K ARAS□TIRMALAR DEG□ERLENDI□RME KOMI□TESI□ BI□LGI□LENDI□RI□LMI□S□ GÖNÜLLÜ OLUR FORMU**

Araştırmanın Adı / Protokol Numarası:

Araştırmanın Konusu:

Kronik Periodontitisli Hastalarda Başlangıç Periodontal Tedaviye Ek Olarak Probiyotik İçeren Striplerin (Prodentis®) Ve Sub-Antimikrobial Doz Doksisiklin Hiklat Doksisiklin Hiklat (Periostat®) Tabletlerin 3 Aylık Kullanımının Klinik Ve Mikrobiyolojik Etkinliğinin Değerlendirilmesi

Araştırmanın Amacı:

Erişkinlerde dişleri çevreleyen çene kemiğinin yatay ve dikey olarak erimesi ve periodontal cep oluşması ile karakterize kronik periodontitisli hastaların tedavisinde başlangıç periodontal tedaviye ek olarak üretici firmanın önerisi doğrultusunda kullanılacak olan probiyotik striplerin ve doksisiklin hiklat içeren tabletlerin 3 aylık kullanımının klinik ve mikrobiyolojik olarak değerlendirilmesi. Araştırmanın Süresi: : 6 ay Araştırmaya Katılan Gönüllü Sayısı:45

Araştırmada İzlenecek Yöntem:

Araştırma Yeditepe Üniversitesi Dişhekimliği Fakültesi Periodontoloji Anabilimdalı'na dişeti hastalığı şikayeti ile başvuran 35–60 yaş arasında klinik ve radyografik bulgulara göre kronik periodontitis tanısı konulacak her bir yarım çenesinde en az 3 tek köklü sondalanabilir cep derinliği ≥5, gingival indeks ≥ 2 olan dişe sahip 45 hasta seçilerek yapılacaktır.

Çalışmaya dahil edilecek bireylerin seçilmesi;

Yeditepe Üniversitesi Dişhekimliği Fakültesi Periodontoloji Anabilimdalı'na başvuran bireyler arasında aşağıdaki kriterler doğrultusunda bireyler seçilecektir.

1) Sistemik olarak sağlıklı olmaları

2) Çalışmadan 6 ay öncesine kadar periodontal tedavi görmemiş ve periodonsiyumu etkileyecek ilaç kullanmamış olmaları

3) Her yarım çenede, radyografik olarak kemik yıkımının gözlendiği, en az bir periodontal bölgede sondalanabilir cep derinliği (SCD) ≥ 5 mm ve gingival indeks (GI; Löe & Sillness 1963) ≥ 2 değerlerine sahip olan en az 3 adet tek köklü dişin bulunması

4) Araştırmaya dahil edilen dişlerde protetik restorasyon bulunmaması

5) Bayan hastaların hamile veya Araştırmaya dahil edilen dişlerde protetik restorasyon bulunmamasıemziren anne olmaması

6) Sigara kullanmamaları

7) Laktoz ve fermente süt ürünlerine alerjik reaksiyon bulunmaması

8) Probiyotik destek ürünü kullanmıyor olmaları

9) Doksisiklin ve türevlerine karşı alerjik reaksiyon bulunmaması

10) Ca^{+2} ve Zn^{+2} içeren ürün kullanmıyor olması

Araştırmanın Planı ve Hasta Grubu

Çalışmaya dahil edilecek hastalara herhangi bir işlem yapılmadan önce periodontal hastalıklar, periodontal hastalığın nedeni olan mikrobiyal dental plak, mikrobiyal dental plaktan korunma yöntemleri, yapılacak periodontal tedaviler ve hastalardan alınacak olan mikrobiyolojik örnekler, probiyotikler ve kullanılacak striplerle ilgili detaylı bilgiler verilerek sözlü ve yazılı onamları alınacaktır. Onamları alınan hastalara ağız hijyen eğitimi, uygun diş fırçası seçimi, diş ipi ve/ veya arayüz fırçası seçimi ve kullanımı öğretilecektir. Diş fırçalarken Modifiye Bass tekniğinin kullanımı anlatılacak ve günde iki kez, sabah ve akşam olmak üzere dişlerin bu teknikte fırçalanmasını takiben arayüz temizliği yapılması istenecektir.

Araştırmaya dahil edilen hastaların periodontal tedavileri tek bir hekim tarafından yapılacaktır. Başlangıç tedavisinden önce ağız hijyen eğitimi verilen hastalar 1 hafta sonra kontrole çağırılacak ve yeterli düzeyde ağız hijyenini sağlayan hastalar rastgele 15'er kişilik 3 gruba ayrılacaktır. Çalışmaya başlamadan 1 hafta önce hastalardan stent hazırlanması için aljinat ile ölçü alınacak, model hazırlanacak ve seri radyografiler hazırlanacaktır. Çalışmaya dahil edilen tüm hastalardan daha önce tespit edilmiş sondalanabilir cep derinliği ≥5 mm ve gingival indeks ≥2 olan dişe sahip iki bölgeden steril paper pointlerle mikrobiyolojik örnekler alınacak ve tüm ağız plak indeksi, gingival indeks, sondalanabilir cep derinliği ve rölatif

ataşman seviyesi değerlerini içeren klinik indeks ve ölçümler yapılıp ağız içi fotoğrafları çekilecektir.

Tüm tedavi gruplarında mikrobiyolojik örnekleri alınan ve klinik ölçümleri yapıldıktan sonra diş yüzeyi temizliği ve kök yüzeyi düzleştirmesi işlemi 1 hafta arayla toplam 2 seans olarak uygulanacaktır. Bu işlemler ultrasonik cihazlarla (piezon® OEM Built- in Kit, EMS, Switzerland) ve Gracey küretlerle (Gracey, SG 3/4, 5/6, 7/8, 11/12, 13 / 14 minifive, SAS $^{3}/_{4}$,Hu – Friedy, USA) gerçekleştirilecektir. Tur ucuna takılan kıl fırça, lastik kon ve temizleme patları ile dişler cilalanacaktır. Bu dönemde hastaların öğretilen mikrobiyal dental plak uzaklaştıma yöntemleri doğru uygulayıp uygulamadıkları da kontrol edilerek gerekli düzeltmeler yapılacaktır. Başlangıç periodontal tedavi dahilinde, oklüzal travmaya neden olacak erken temas noktaları saptanıp, bu alanlar ortadan kaldırılacaktır, çürük dişler mevcutsa, tedavileri gerçekleştiricilecektir. Ayrıca endodontik konsültasyon sonrasında tespit edilen devital dişler tedavi edilecektir. Çekim yapılacak dişler araştırmaya dahil edilmeyecektir.

1. gruba diş yüzeyi temizliği ve kök yüzeyi düzleştirmesi ile beraber *Lactobacillus reuteri* (Prodentis®) içeren strip 3 ay boyunca sabah ve akşam birer tane olmak üzere günde 2 kez kullandırılacaktır. 2. gruba diş yüzeyi temizliği ve kök yüzeyi düzleştirmesi ile beraber sub-antimikrobial doz doksisiklin hiklat (Periostat®) içeren tablet 3 ay boyunca sabah ve akşam birer tane olmak üzere günde 2 kez kullandırılacaktır. 3. gruba sadece diş yüzeyi temizliği ve kök yüzeyi düzleştirmesi uygulanacaktır. 3. ayda klinik ve mikrobiyolojik örneklemeler tekrarlanacaktır.

Araştırmada Kullanılacak Klinik İndeks ve Ölçümler

Araştırmada kullanılacak indeks ve ölçümlerin birbirini olumsuz yönde etkilememeleri için belirli bir düzen içinde yapılacaktır. Klinik ölçümler, uygulanacak tedavinin içeriği hakkında bilgisi olmayan bir hekim tarafından 0. Gün 3 ve 6. Ayda yapılacaktır. Bu işlemler sırasında, muayene sondu ve 0.4 mm çapında 15 mm'lik periodontal sonda (*Universitiy of North Carolina PCPUNC15, Hu-Friedy Ins. Co., ABD*) kullanılacaktır. Periodontal sondanın doğru yerleştirilebilmesi ve tüm ölçüm dönemlerinde hataların en aza indirgenmesi amacıyla sabit rehber noktaları bulunan hastaya özel akrilik stentler yapılacaktır. Bu stentler üst ve altçene için ayrı ayrı dişlerin oklüzal yüzlerini ve kuronal 1/3 ünü kaplayacak şekilde hazırlatılacaktır.

Plak indeksine göre;

0- Gözle bakıldığında ve sondla muayene edildiğinde dişeti kenarında mikrobiyal dental plak yoktur.

1- Dişeti kenarında mikrobiyal dental plak gözle zor seçilirken sadece sonda ile muayenede sondanın ucunda mikrobiyal dental plak gözlemlenmektedir.

2- Dişeti bölgesinde gözle görülebilen ince ve orta düzeyde mikrobiyal dental plak vardır, interdental bölge tamamen dolmamıştır.
3- Dişeti kenarında, dişeti oluğu içerisinde ve komşu diş yüzeyinde fazla miktarda mikrobiyal dental plak vardır, interdental bölge tamamen dolmuştur.

Gingival indeks:

Her dişin meziyo-bukkal, distobukkal ve mid-lingual olmak üzere 4 yüzünde dişetinin renk, ödem, kıvam ve kanama durumuna göre 0-3 arasında değer verilecektir. Bu indekse göre:

0- Normal dişeti
1- Dişetinde hafif iltihap gözlenmektedir, hafif renk değişimleri ve ödem vardır, ancak sondalamada kanama yoktur.
2- Orta derecede iltihap görülür, dişetinde kırmızılık, ödem ve parlaklık vardır, sondalamada kanama mevcuttur.
3- Şiddetli iltihap, belirgin kırmızılık ve ödem vardır, ülserasyon olabilir. Spontan kanamaya eğilim söz konusudur.

Sondalamada kanama:

Sondalanabilir cep derinliği ölçüldükten sonra dişlerin çevresindeki 4 noktasından (meziyo-bukkal, mid-bukkal, mid-lingual, distobukkal) kanama var (+) ya da yok (-) şeklinde kaydedilecektir.

Sondalanabilir cep derinliği:

Akrilik oklüzal stentler ve üzerinde frezle açılan oluklar rehberliğinde, periodontal sonda cep içerisine yerleştirilecektir. Cep tabanı ile dişeti kenarı arasındaki mesafe ölçülecektir. Her dişin bukkal, oral, hem bukkal hem de oral tarafından meziyal ve distal köşe açıları olmak üzere toplam 6 noktasından ölçüm yapılacaktır.

Rölatif Ataşman Seviyesi

Oklüzal stentler üzerinde sondalanabilir cep derinliği ölçümlerinin yapıldığı noktalardan, stent apikal kenarı sabit rehber noktası alınarak cep tabanı ile stent kenarı arasındaki mesafe

kaydedilecektir. Her dişin bukkal, oral, hem bukkal hem de oral taraftan olmak üzere toplam 6 noktadan ölçüm yapılacaktır.

Mikrobiyolojik Kültür Yöntemi

Mikrobiyolojik örnekler her hastanın önceden tayin edilmiş sondalanabilir cep derinliği ≥5, gingival indeks≥2 olan periodontal cep bölgelerinden tedavi öncesi ve tedavi sonrası 3 ve 6. Ayda alınacaktır. Örneğin alınacağı bölgedeki diş yüzeyinden supragingival plak sond ve gaz tampon yardımı ile uzaklaştırılıp diş yüzeyi hava spreyi ile kurutulacaktır. Kanamanın olmamasına dikkat edilerek steril 30 numaralı paper point (*Meta Biomed Co., Korea*.) periodontal cep içerisine hafif direnç hissedilene kadar yerleştirilip 10 sn beklenecektir. Alınan subgingival mikrobiyolojik örnek aseptik koşullarda 4,5 ml phosphate- buffered saline (*phosphate-buffered saline, PBS tablet, Medicago AB, Uppsala İsveç*) içeren tüplere aktarılacaktır. Homojen dağılım sağlamak amacıyla tüpler 30 sn süreyle vorteks karıştırıcıda karıştırılacak ve aynı tampon içerisinde on katlı sulandırmalar yapılacaktır. Uygun sulandırmalardan (10^{-1}, 10^{-2} ,…..10^{-6}) 0.1 ml'lik 2 ayrı hacim alınarak % 0.0005 hemin (*Sigma 33H0829, Sigma Chemical Co., ABD*), %0.00005 menadion (*Sigma 123H2617, Sigma Chemical Co., ABD.*) ve %5 oranında koyun kanı ile zenginleştirilmiş trypticase soy agar dökülen 2 petri kutusuna steril yavrulu tüp yardımıyla homojen olarak yayılacaktır. Birinci besiyeri anaerop koşullarda (Gas Pak Jar) (*Oxoid Ltd., İngiltere*.) 37ºC'de 7-10 gün, diğeri ise %5 CO_2 içeren ortamda (CO_2 Gen) (*Oxoid, CO_2 Gen, Oxoid Ltd., İngiltere*) 37 ºC'de 5 gün bekletilecektir.Besiyerlerinde üreyen mikroorganizmaların kolonileri sayılacak, oksijene karşı durumlarına göre fakültatif anaerop ve zorunlu anaerop olmak üzere 3 grup mikroorganizmanın 1 ml'deki sayısı ve oranı kaydedilecektir.

Alternatif Tedavi veya Girişimler:

Araştırma Sırasında Karşılaşılabilecek Riskler: Literatürde uygulanacak yöntem ile ilgili herhangi bir riskli durum tespit edilmemiştir.

Araştırma İlacının Olası Yan Etkileri: Araştırmada ilaç kullanımı yoktur.

Araştırma Süresince 24 Saat Ulaşılabilecek Kişi Adı / Soyadı / Telefonu:

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Bilgilendirilmiş Gönüllü Olur Formundaki tüm açıklamaları okudum. Bana, yukarıda konusu ve amacı belirtilen araştırma ile ilgili yazılı ve sözlü açıklama aşağıda adı belirtilen hekim tarafından yapıldı. Araştırmaya gönüllü olarak katıldığımı, istediğim zaman gerekçeli veya gerekçesiz olarak araştırmadan ayrılabileceğimi ve kendi isteğime bakılmaksızın araştırmacı tarafından araştırma dışı bırakılabileceğimi biliyorum.
Söz konusu araştırmaya, hiçbir baskı ve zorlama olmaksızın kendi rızamla katılmayı kabul ediyorum.

Gönüllünün Adı / Soyadı / İmzası / Tarih

Açıklamaları Yapan Kişinin Adı / Soyadı / İmzası / Tarih

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