T.C.

YEDITEPE UNIVERSITY
INSTITUTE OF HEALTH SCIENCES
DEPARTMENT OF PERIODONTOLOGY

PLAQUE INHIBITORY EFFECT OF HYALURONAN-CONTAINING HYDROGEL MOUTHWASH IN A 4-DAY NON-BRUSHING MODEL

DOCTOR of PHILOSOPHY THESIS

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ISTANBUL-2018

APPROVAL

Institute: Yeditepe University Institute of Health Sciences

Programme: Periodontology Doctorate Programme

Title of the Thesis : Plaque Inhibitory Effect of Hyaluronan-Containing Hydrogel Mouthwash in a 4-Day Non-Brushing Model

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Examination Date: 02.07.2018

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This thesis has been deemed by the jury in accordance with the relevant articles of Yeditepe University Graduate Education and Examinations Regulation and has been approved by Administrative Board of Institute with decision dated 06.07.2018..... and numbered 2018/12-09

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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.

02.07.2018

BEGUM ATALAY, DDS.

ACKNOWLEDGEMENTS

First and foremost, I would like to express my gratitude to my supervisor and programme director Prof. Dr. Bahar Eren Kuru for her guidance and mentorship throughout my PhD education and for sharing her expertise in the field of Periodontology,

My sincere thank goes to Assoc. Prof. Dr. Hare Gursoy for her valuable influence, vision and patience throughout my education life. She had remarkable contributions to my life and to this research.

I have been grateful to Dr. Ebru Ozkan Karaca for offering me her clinical experience and advices in my postgraduate education.

I will always be thankful for my friend Dr. Ogul Leman Tunar for her support, advices and friendship throughout my education life.

I also thank to Dr. Gizem İnce Kuka for her friendship and support,

I also would like to send my thanks to my colleque Nesrin Al-Naihoum for standing by me, since the very beginning of my PhD.

Special thanks to my all colleagues in the department, who had been there, whenever I needed.

Last but not the least, I would like to thank my family: to my dear father Osman Atalay for his endless support and encouragement throughout my life, and to my dear mother, Mutlu Atalay for her endless patience and optimism, which kept me alive for the hard times.

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LIST of SYMBOLS and ABBREVIATIONS

MDB	Microbial Dental Biofilm
CHX	Chlorhexidine
HIV	Human Immunodeficiency Virus
HA	Hyaluronan, Hyaluronic Acid
EO	Essential Oil
GAG	Glycosaminoglycan
ECM	Extracellular Matrix
GBR	Guided Bone Regeneration
GCF	Gingival Crevicular Fluid
ADA	American Dental Association
CPC	Cetylpyridinium Chloride
SLS	Sodium Lauryly Sulfate
DW	Distilled Water
PI	Plaque Index
GI	Gingival Index
SD	Standart Deviation
VAS	Visual Analogue Scale
SQ	Satisfaction Questionaire
D1	Day 1
D5	Day 5
QHPI-s	Quigley Hein Plaque Index System
MGI	Modified Gingival Index
TNF	Tumor Necrosis Factor
ROS	Reactive Oxygen Species
MW/A	Mouthwash A
MW/B	Moutwash B
MW/C	Mouthwash C
IL	Interleukin
NETs	Neutrophil Extracellular Traps
SEM	Scanning Electron Microscope
HMW-HA	High Molecular Weight Hyaluronan
LMW-HA	Low Molecular Weight Hyaluronan

ABSTRACT

Atalay, B. (2018). Plaque-inhibitory Effect of Hyaluronan-containing Hydrogel Mouthwash in a 4-day Non-Brushing Model. Yeditepe University Institude of Health Sciences, Department of Periodontology, Doctor of Philosophy Thesis, Istanbul.

The primary prevention and treatment of plaque-induced gingivitis is obtained by the combination of mechanical and adjunctive chemical supragingival biofilm control. Despite being gold standart anti-plaque chemical agent, many reported adverse effects of chlorhexidine (CHX) make scientists search for new agents to combat biofilms as effective as CHX. Hyaluronan (HA) is a naturally occuring polysaccharide, which induces wound healing with its antiinflammatory, antioxidant, bacteriostatic and viscoelastic properties. In recent years, HA-based biomaterials have been started to use in oral care products. The objectives of this study were to evaluate the plaque inhibitory effect of a HA-containing mouthwash (test) in comparison with CHX-containing mouthwash (positive control) and a distilled water containing mouthwash (negative control) in a 4- day non-brushing model and also to evaluate patients' experience and adverse effects of the mouthwashes after their usage. Thirty-three systemically and periodontally healthy subjects were included in this short-term, randomized, double-blind, cross-over clinical study. These individuals were randomly and equally assigned to one of the following treatment groups after professional supra-gingival prophylaxis: Treatment Group A (n=11), Treatment Group B (n=11), Treatment Group C (n=11). Subjects were asked to stop their mechanical oral hygiene habits for 4 days and instructed to use only the mouthwash that was allocated to them. The outcome variables were the plaque index (PI) as the primary outcome variable, gingival index (GI) and gingival crevice fluid (GCF) volume as the secondary outcome variables. In addition, treatment satisfaction questionaire was performed at the end of each experimental period in order to evaluate the adverse effects of the treatment products. CHX-containing mouthwash showed significant reductions in plaque accumulation compared to HA-containing mouthwash and the distiled water. However, no significant differences were detected between HA and CHX containing mouthwash treatment groups in terms of GI values ($p>0.05$). Inter-treatment comparison of the mean increases of GCF volume (µl) were not detected statistically significant between D1 and D5. HA-containing mouthwash well accepted and preferred when compared to the CHX mouthwash.

Keywords: chlorhexidine, hyaluronan, non-brushing model

ABSTRACT (Turkish)

Atalay, B. (2018). Hyaluronik Asit içeren Hidrojel ağız gargarasının, Plak İnhibisyonu Üzerindeki Etkisinin 4 Günlük Plak Akümülasyon Modelinde Klinik Olarak Değerlendirilmesi. Yeditepe Üniversitesi Sağlık Bilimleri Enstitüsü, Periodontoloji Anabilim Dalı, Doktora Tezi, İstanbul.

Plağa bağlı dişeti hastalıklarından primer korunma, mekanik ve kimyasal supra-gingival biofilm kontrolü ile elde edilmektedir. Klorheksidin altın standart olarak kabul edilen bir antiplak ajan olmasına rağmen, rapor edilen istenmeyen etkileri araştırmacıları klorheksidine alternatif olacak, fakat kendisi kadar etkili yeni ajanların arayışı içine sokmuştur. Hyaluronik asit, anti-inflamatuar, antioksidan, bakteriyostatik ve viskoelastik özellikleri ile yara iyileşmesini uyaran doğal yolla oluşan bir polisakkarittir. Son yıllarda hyaluronik asit içeren biyomateryaller ağız bakım ürünlerinde kullanılmaya başlanmıştır. Bu çalışmada, 4 günlük plak akümülasyon modelinde hyaluronik asit içeren ağız gargarasının plak önleyici etkisinin, distile su ve klorheksidin içeren ağız gargarası ile klinik olarak kıyaslanması, hasta memnuniyetinin ve gargara kullanımı sonrasında meydana gelen yan etkilerinin değerlendirilmesi amaçlandı. Kısa dönemli, randomize, çift-kör, cross-over klinik çalışmaya, sistemik ve periodontal olarak sağlıklı 33 gönüllü birey dahil edildi. Bu bireyler, kendilerine uygulanan profesyonel supragingival profilaksi işlemi sonrasında, her biri rastgele seçilmiş 11 kişiden oluşan 3 tedavi grubuna ayırıldı. Bireylerden mekanik ağız hijyen alışkanlıklarını kesmeleri ve 4 gün süreyle sadece gargara kullanmaları istendi. Çalışmada plak indeksi, gingival indeksi, dişeti oluğu sıvısı hacmi değerlendirildi. Klorheksidin içeren ağız gargarasının distile su ve hyaluronik asit içeren ağız gargarasına göre plağı önemli ölçüde azalttığı tespit edildi ($p<0.05$). Fakat 3 tedavi grubu arasında gingival index parametresi açısından herhangi bir fark gözlenmedi ($p>0.05$). Dişeti oluğu sıvısı hacmindeki artışın gruplar arası kıyaslanmasında istatistiksel olarak anlamlı farklılık tespit edilmedi ($p>0.05$). Hyaluronik asit içeren ağız gargarası klorheksidin içeren ağız gargarasına kıyasla bireyler tarafından daha kolay kabul edilen ve daha çok tercih edilen gargara oldu.

Anahtar kelimeler: klorheksidin, hyaluronik asit, plak akümülasyon modeli

1. INTRODUCTION and PURPOSE

Periodontal diseases induced by dental plaque, mainly classified as gingivitis and periodontitis, are considered as the most prevalent diseases of the oral cavity, may accompanied by dental caries (1). Dental plaque is considered as the primary etiological factor of these diseases and defined as the microbial community that develops on the tooth surfaces in a form of structurally and functionally organized species rich microbial dental biofilm (MDB). MDB's are biologically active structures and, release toxic by-products that initiates an inflammatory host-response, resulting in inflammation of the gingival tissues, the so-called gingivitis (2). If gingivitis left untreated and the accumulation of MDB allowed, the advanced form of the disease, periodontitis, occurs in susceptible patients. Considering that gingivitis and periodontitis are the continuum of the same inflammatory disease, prevention of biofilm formation has primary importance for the prevention, occurance and treatment of these diseases (3).

The current preventive and treatment strategies in the management of these disases are mainly focused on the primary etiological factor, MDB, and pathogenesis of these conditions. Almost 50 years of experimental research, clinical trials in different geographical and social settings have confirmed that effective removal of MDB is essential to dental and periodontal health (4). MDB control is basically achieved by the combination of compliance with daily self-performed oral care either using mechanical devices and/ or adjunctive chemical formulations and by professional MDB and calculus removal at regular dental visits (5). Self-performed oral care with mechanical devices, including toothbrushes and interdental cleaning aids, are currently the most common methods to remove MDB (6). However, in a systematic review of the effectiveness of self-performed mechanical plaque removal in adults with gingivitis showed that the quality of the mechanical plaque control was not sufficiently effective in reducing gingivitis (7). Reasons for insufficient mechanical plaque removal are many and include noncompliance with the frequency of brushing time, inadequate manual dexterity, inappropriate brushing tecknique of the patients and some additional circumstances which makes plaque removal nearly impossible with mechanical devices such as patients with intermaxiller fixed orthodontic appliances or after periodontal and dental surgeries (8, 9). In order to overcome these limitations, adjunctive chemical biofilm control agents are formulated to control supra-gingival MDB in a form of mouthwashes, gels and dentrifices (10, 11). According to their mechanism of action on MDB, these agents can

be categorized as antimicrobial agents, plaque reducing/inhibitory agents, antiplaque agents and antigingivitis agents (12). These chemical agents include bisbiguanides, quaternary ammonium compounds, phenolic agents, oxygenating agents, metal ions, natural products and miscallenous agents (13).

Chlorhexidine (CHX) gluconate, a bisbiguanide, is a gold standart antiplaque agent, which has long-lasting bacteriostatic and bactericidal effects on plaque biofilms depending on its concentration. It has broad spectrum antimicrobial action against microorganisms including, Gram negative and Gram positive bacteria, fungi and yeasts including *Candida* species and some viruses including Hepatitis B virus and Human Immunodeficiency Virus (HIV) (14). However, many adverse effects have been reported attributed to its effects at high concentrations (15). Therefore, scientists are searching for new agents to combat plaque biofilm formation on tooth surfaces either by reducing the concentration of CHX or by adding some chemical compounds which are as effective as CHX's anti-plaque effect (9).

Hyaluronan, also known as hyaluronic acid (HA), is one of the recent chemical agent under investigation, is a negatively charged and non-sulphated linear glycosaminoglycan (GAG) found naturally in vertebrate organs, fluids, connective, epithelial and neural tissues. In humans, HA is abundant in the vitreous of the eye, the umbilical cord, synovial fluid, heart valves, skin, and skeletal tissue. It is a ubiquitous component of the vertebrate exracellular matrix (ECM) and participates in a wide variety of physicochemical and biological processes. HA is biocompatible, non-immunogenic, biodegredable, viscoelastic and hygroscopic, and these properties make it a preferable biomaterial for medical and pharmaceutical applications (16). HA enhanses regeneration, stimulates osteoinduction and involves in osseointegration (17). It has inductive wound healing and adhesive properties and possesses antiinflammatory properties when applied topically. Up to date, HA-based biomaterials, have been used in osteoartritis, in ophtalmotology as a viscosupplementation agent, in esthetic medicine and recently in oral-car .

As a consequence of its non-toxicity, biocompatibility and numerous biochemical and physicochemical properties, the use of HA-based biomaterials, applied topically to inflamed periodontal sites would offer beneficial effects in modulating and accelerating the host response (18). In the field of periodontology, HA has been used as an antimicrobial agent adjunct to scaling and root planing in the treatment of gingivitis and periodontitis, as a sealing agent for both implant and sins lift procedures for faster healing

and to reduce the patients discomfort during the postoperative period, as a bone regenerating agent in periodontal bony defects, in Guided Bone Regeneration (GBR), in peri-implant maintenance of immediate function implants and as an autologous cell hyaluronic acid graft for gingival augmentation in mucogingival surgeries, and finally in the treatment of oral ulcers (19, 20). It is available as spray form, gel form and mouthwash form, also available in various concentrations, range between 0.025% and 0.8% As it is a newer drug, fewer studies has been carried out using HA-containing mouthwash in a 4- day non-brushing study (21).

The aim of the present study was to clinically evaluate 0.025% HA-containing mouthwash in comparison to 0.2% CHX-containing mouthwash and distilled water in terms of plaque inhibition, gingival inflammation, volumetric changes in gingival crevicular fluid (GCF), satisfaction questionaire responses, adverse effects in a 4-day non-brushing model, using a double blind, randomized, Latin-square controlled clinical trial.

2. LITERATURE REVIEW

Periodontal diseases are initiated as dental plaque-induced gingival inflammation and without treatment, in susceptible patients, may lead to the periodontitis with the contribution of risk factors. Plaque-induced gingival inflammation, gingivitis, is the physiologic response of the tissues to the accumulation of dental plaque at or near gingival margin (2). Periodontitis is a complex polymicrobial infection, leading to tissue destruction as a consequence of the perturbation of the homeostasis between the subgingival microbiata and the host defences in susceptible individuals. These diseases are a continuum of the same inflammatory disease and prevention of the gingival inflammation by disruption of dental plaque accumulation can prevent the occurance of these diseases (22).

2.1. Microbial Dental Biofilm

Microbial Dental Biofilm (MDB) is a clinically structured, resilient yellow greyish substance that persistantly adheres to intra-oral hard surfaces, which can not be removed by rinsing or the use of spreys. It is defined as a structurally and functionally organized diverse multi-species microbial community that forms on tooth surfaces which capsulated in an extracellular matrix consisting of organic and inorganic materials derived from saliva, gingival crevicular fluid (GCF), and bacterial products (1). It is composed of between approximately 400 and 1,000 microbial species in a human microbiome of many thousands of species (23).

MDB's are complex three-dimentional structures that shows a stratified composition of a multilayered accumulation of bacterial morphotypes which has polymer-containing channels that link the plaque/oral environment interface to the tooth surface embedded in a exopolysaccharide matrix (Figure 2.1.) (24). MDB is mainly classified as supra-gingival and sub-gingival biofilm which differs in their site specificity, structure, microbial composition and metabolism. Supra-gingival biofilm is located either "at" or "above" the gingival margin and when in direct contact with the gingival margin, it is referred as marginal plaque. Gram positive cocci and short rods predominate at the tooth surface, whereas Gram negative rods and filaments as well as spirochetes predominate in the outer surface of the mature plaque mass. The most viable bacteria are

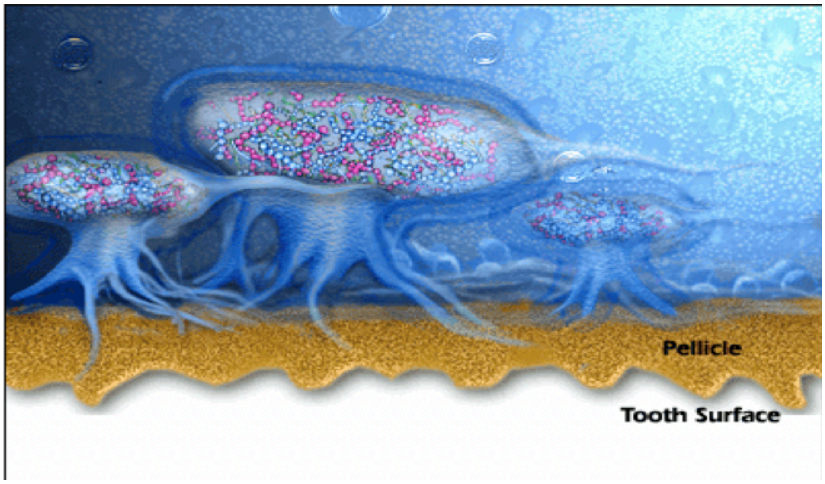

Figure 2.1. The Graphical Structure of Microbial Dental Biofilm (24).

present at the center of its architecture which produce various different exo-polymers that forms extracellular matrix and prevent the penetration of therapeutic agents (25). Sub-gingival plaque is found below the tooth and the gingival sulcus epithelium (26). Histological sections of human sub-gingival plaque samples revealed a complex organization of attached microorganisms which exist as distinct tooth-associated and epithelial cell-associated biofilms, with the possibility of a less dense zone of organisms between the two (27). These regions may differ in microbial composition, physiological state and, consequently, in their response to antimicrobial treatment.

The diseases of the periodontium is associated by the site-specificy of plaque. Marginal plaque is important in the initiation and development of gingivitis. Supra-gingival plaque and tooth-associated sub-gingival plaque have critical importance in calculus formation and root caries, whereas tissue-associated sub-gingival plaque is decisive in the tissue destruction that characterizes different forms of periodontitis .

The composition of MDB differentiates in response to changes in the local environment and lifestyle, over time, on different anatomical surfaces, in people of different ages, from different countries and diets and with deficiencies in their host defences and following various therapies (28). These discrepencies can affect the microbial interactions within these oral communities and determine whether the relationship between the oral microbiome and the host is symbiotic or potentially damaging (29, 30).

The formation of MDB on a tooth surface is a dynamic and complex process that

follows several individual phases (Figure 2.2). In oral cavity, all surfaces including hard and soft tissues are layered with an organic conditioning film known as the acquired pellicle (18). The acquired pellicle on tooth surfaces is composed of a number of peptides, proteins, glycoproteins and other molecules, functioning as adhesion sites for bacteria (31). By the flow of saliva, microorganisms are transported passively to the surface of tooth (21). Immediately after cleaning or following initial exposure to the oral environment, acquired pellicle molecules are adsorbed to the tooth surface within seconds and remain functional (32). This functional acquired pellicle modifies the biologic and chemical properties of the tooth surface, and its composition directly influences the pattern of succeeding microbial colonization. The spesific interaction between microbial cell surface adhesin molecules and receptors in the acquired pellicle determines the association of bacterial cell with the tooth surface. The first bacteria to colonize the surface of the tooth by this acquired pellicle are mostly Gram-positive, facultative cocci, mainly *Streptoccoccus species* and coccobacilli mainly *Actinomyces*. This early colonization is transient and bacteria holds reversibly near to the surface by weak, long range, physicochemical forces between the electrical charge of the molecules on the pellicle-coated surface and those on the cell surface (33). This attachment is mediated by proteoglycans covering the cell wall, as well as proteins in fimbria and pili. Once attached, the early colonizers start to multiply and the thickness of the plaque increases with bacterial multiplication. During the first day, the tooth surface is gradually covered by multiplying bacteria. They begin to grow away from the teeth, in the form of columnar microbial colonies that are closely packed and compete for space and nutrients. Around day 3, corncob formations are produced by the aggregation of filamentous bacteria to the previously formed coccoid plaque. This competitive growth continues approximately 1 week. During this time, filamentous bacteria begin to penetrate to the coccoid plaque from the surface, gradually replacing the coccoid microbiata with a predominantly filamentous microbiota. This process may continue for approximately 2 or more weeks. As the biofilm develops, adhesins on the cell surface of more fastidious secondary colonizers, such as obligate anaerobes, bind to receptors on bacteria that are already attached by a process termed co-adhesion or co-aggregation (34, 35). *Fusobacterium nucleatum* is a key organism in plaque biofilm development. These species acts as an

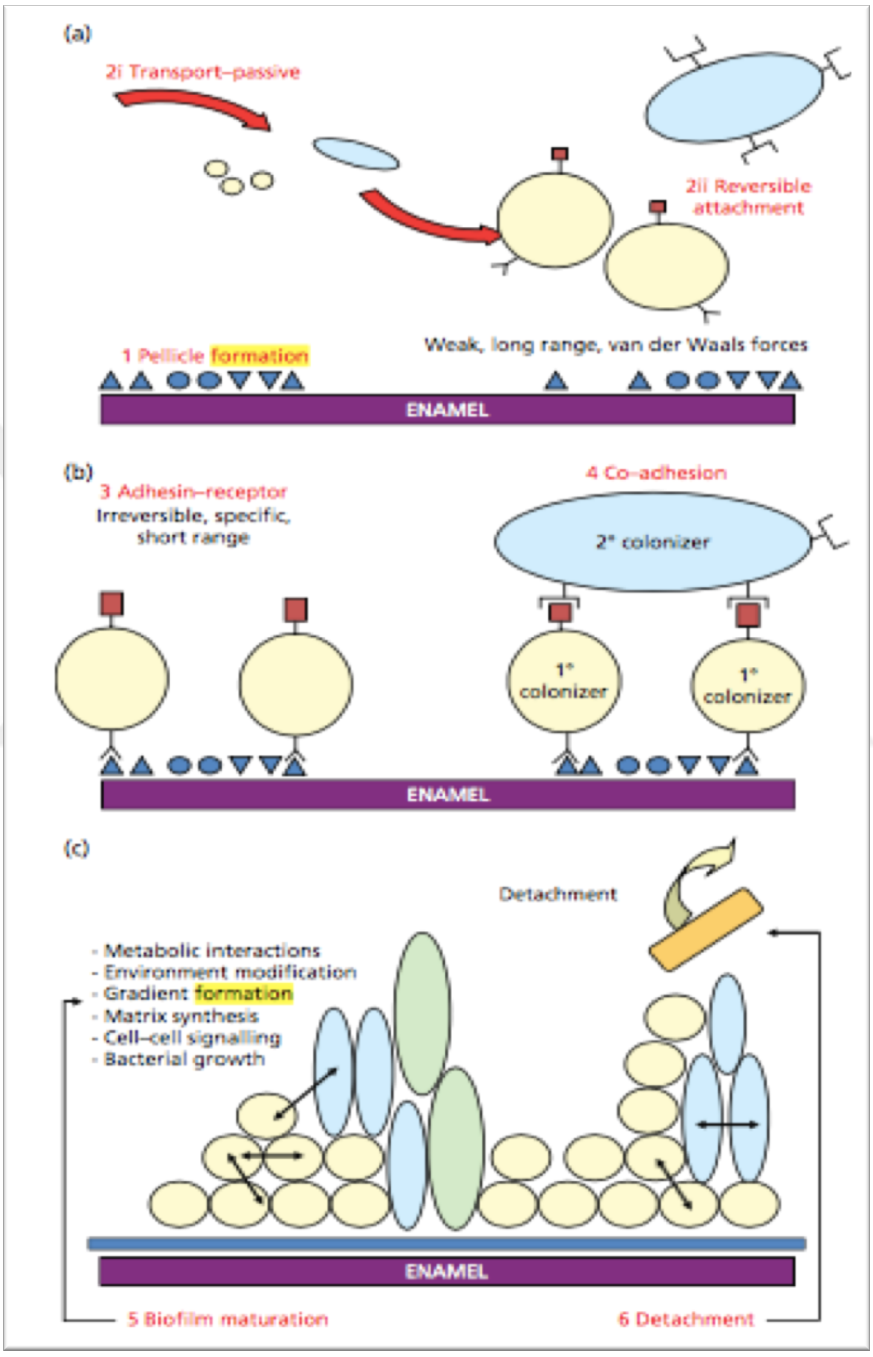

Figure 2.2. The Stages of Microbial Dental Biofilm Formation (36).

important bridging organism between early and late colonizing species. Co-adhesion may help ensure that bacteria co-locate with other organisms with complementary metabolic functions (37). Both Gram positive and Gram negative bacteria have several families of surface proteins that can function as adhesins (38). As the biofilm matures, there is continued synthesis of exo-polymers to form an extracellular matrix. The matrix is physically important as being part of the scaffolding that determines the structure of biofilms, and also biologically active as it retains water, nutrients and key enzymes within the biofilm. Colonization of microorganisms in a biofilm structure is coordinated by cell-cell communication and /or cross-communicating between Gram positive and Gram negative bacteria by quorum sensing ability of the biofilm matrix. In this way, the micro-flora remains relatively stable over time. This stability results from a dynamic balance arising from numerous microbial community interactions, including both synergism and antagonism. Thus, microbial community interaction in a biofilm structure provides a broader habitat range for growth, causes an increased metabolic diversity and efficiency, an enhanced resistance to environmental stress and antimicrobial agents and host defenses, and an enhanced ability to cause disease (26).

2.2. Pathogenesis of Plaque-Induced Gingivitis

Plaque-induced gingivitis is the inflammation of the gingival tissues resulting from the accumulation of bacteria at or near the gingival margin (39). Clinically, plaque-induced gingivitis is characterized by, presence of marginal MDB; change in gingival color; change in gingival contour; sulcular temperature change; increased gingival crevicular fluid (GCF) and bleeding upon probing. The initial changes from health to -induced gingivitis may not be detectable clinically, but as plaque-induced gingivitis progresses to more advanced forms of this disease, clinical signs and symptoms become more obvious (40). The strength of the clinical signs and symptoms may vary among individuals as well as among sites within a dentition (41).

The pathogenesis of plaque-induced gingivitis has been separated into the initial, early, and established stages, each with characteristic clinical and histopathological features. The “initial” lesion occurs 2–4 days following the beginning of plaque accumulation. At this time, the gingiva appears to be healthy normal gingiva clinically. Pathohistologically, acute signs characteristic of an initial lesion are visible. It is characterized by the formation of edema with increase in gingival crevicular fluid (GCF) flow, an accumulation of polymorphonuclear neutrophils (PMNs), and loss of

perivascular collagen. *Streptococci* are among the first organisms to colonize the acquired pellicle as plaque develops. These organisms produce a range of enzymes and metabolic end products, which increase the permeability of the junctional epithelium, allowing both the ingress of further bacterial products and at the same time the outflow of GCF. At this early stage, the GCF is essentially the same as interstitial fluid, but contains many serum proteins, including all the components necessary for the activation of complement (40). Lipoteichoic acid and peptidoglycans, which are components of the cell wall of these early colonizers, are capable of activating "alternative pathway'' in the gingival sulcus and results in the production of the ''anaphylatoxins'', which in turn flow back into the tissues. Once in the tissue, these anaphylatoxins lead to the release of vasoactive amines from resident mast cells. In turn, these vasoactive amines lead to an increase in vascular permeability and the formation of edema, one of the hallmarks of inflammation. Mast cells also release preformed cytokines, including tumor necrosis factor-alpha (TNF-α), which results in the expression of adhesion molecules by endothelial cells and the subsequent sticking and migration of PMNs into the gingival tissues. While activation of the alternative complement pathway is essential for the vascular responses, bacterially-derived chemotactic substances together with C5a are responsible for the migration of PMNs into the gingival sulcus. Once in the gingival sulcus, the PMNs are unable to phagocytose the bacteria, which are beginning to form a biofilm and as such are firmly adherent to the tooth surface. In this situation, the PMNs disgorge their lysosomal contents into the gingival sulcus in what has been termed "abortive phagocytosis". These lysosomal enzymes can then return into the tissues and contribute to the local destruction of connective tissues. In addition, PMNs release structures called neutrophil extracellular traps (NETs), which can trap and kill microbial pathogens. NETs are released during a form of pathogen-induced cell death, recently called NET'osis, that differs from apoptosis and necrosis and represents one of the first lines of defense against pathogens. *In vivo* both dead and viable PMNs can release NETs, which in turn can be associated with severe tissue damage. In addition, a variety of pro-inflammatory stimuli, all of which can be found in the gingival sulcus, such as lipopolysaccharide (LPS), interleukin-8, TNF, as well as the streptococcal M protein can all induce NET formation. While NETs have been described in periodontitis, it is likely that they are also formed in this initial lesion stage of gingivitis and then persist through all stages of gingivitis and periodontitis. Other cell types, such as eosinophils and mast cells, are also able to release extracellular traps These mast cell extracellular traps appear to be released in response to the same factors that lead

to NET release from PMNs. Within the gingival sulcus, PMNs also produce and release a variety of cytokines including IL-1, the IL-1 receptor antagonist, and high levels of IL-17. IL-17 in turn induces the production of IL-8 by sulcus epithelial cells. IL-8 is not only a very strong chemo-attractant for PMNs, but as stated earlier, is also a strong stimulus for NET formation, thus establishing a positive feedback loop in an attempt to contain the developing bacterial infection. Indeed, it is highly likely that the role of IL-17 in periodontal disease is a protective one in that it maintains the PMN barrier in the gingival sulcus. It is well established that loss of this barrier, either due to an absence of PMNs (such as agranulocytosis or cyclic neutropenia) or a defect in their function (either chemotactic or phagocytic), leads to severe and rapid progression of periodontal destruction. At this initial stage however, the lesion occupies no more than 5–10% of the connective tissues, and is still not evident clinically (42)**.** The so-called "early" lesion develops after approximately 4–7 days of plaque accumulation. At this stage the nature of the developing lesion changes from one consisting primarily of PMNs to one with increased numbers of lymphocytes and macrophages. Vascular changes become more pronounced with the opening of previously dormant capillary beds, the formation of post-capillary venules, increased vascular permeability, and the devel- opment of perivascular inflammatory infiltrates. As a result, there is a net increase in the flow of fluid into the affected gingival tissues, and a subsequent increase in the flow of GCF. The nature of the GCF at this stage changes from that of interstitial fluid to that of an inflammatory exudate, in other words edema. An increase in the permeability of the sulcular and junctional epithelia, as a result of widening of the intercellular spaces between the epithelial cells, allows increased ingress of bacterial products into the gingival tissues and escalation of the inflammatory response (43). Initially, the lesion develops as small perivascular infiltrates which progressively increase in size and coalesce such that at around day 12–21 following the beginning of plaque accumulation the lesion becomes clinically evident. By day 21, lymphocytes make up 70% of the infiltrate and although there is a four-fold increase in PMN numbers within the junctional epithelium PMNs and plasma cells make up <10% of the total infiltrate As with the initial lesion, the release of cytokines such as TNF-α and IL-17 from mast cells and PMNs undergoing NETosis leads to an increase in cell adhesion molecules, such as endothelial cell leukocyte adhesion molecule-1 and intercellular adhesion molecule-1, which together with an increase in IL-8 production by the epithelial cells help to establish a fast flow of PMNs through the junctional epithelium

and into the gingival sulcus where they form a barrier against plaque microorganisms. Although the infiltrated area remains fairly localized at this stage, up to 60–70% of collagen within the infiltrated zone is degraded (40).

2.3. Prevention of Periodontal Diseases

Prevention is defined as an act which results in keeping something from happening or making something impossible to happen. In terms of periodontology, prevention is related to a set of various actions which ultimately prevent or control the occurance, progression and duration of the disease (44).

In the literature, following distinctive preventive measures are made between primary, secondary and tertiary prevention levels (45);

- Primary prevention is defined as the pre-pathologic or pre-clinical stage which aims to prevent the onset of the disease with the concept of health promotion and protection strategies. At this stage, preventive measures should be applied to eliminate or control both etiologic and risk factors to prevent the development of gingival inflammation and maintain healthy gingival tissues.
- Secondary prevention refers to the early stage of the disease and is based on early diagnosis, prompt treatment to reverse the disease process and to restore health. Secondary prevention applies to a condition such as gingivitis, an inflammation limited to the gingival tissues caused by plaque accumulation. The intention is to stop and reverse the disease process by removing the etiologic or causal factors. Clinical experimental studies and clinical experience showed that the inflamed gingival tissue may revert to normal following adherence to strict oral hygiene measures.
- Tertiary prevention applies to disease conditions. It aims at limiting sequels, rehabilitating functions, and maintaining health and preventing relapse following active treatment. Supportive periodontal care or periodontal maintenance may serve as an example to illustrate this concept. It is designed to assist the patient in maintaining oral health and prevent the recurrence or progression of disease in patients who have been previously treated for periodontitis or peri- implant disease (46).

2.4. Primary Prevention of Plaque-Induced Gingivitis

At present, both primary prevention of gingivitis and primary and secondary prevention of periodontitis are based on the achievement of sufficient plaque removal. Almost 50 years of experimental research, clinical trials in different geographical and

social settings have confirmed that effective removal of dental plaque is essential to dental and periodontal health (4, 44). Primary prevention strategies include educational interventions for periodontal diseases and related risk factors; regular, supra-gingival biofilm control either using mechanical devices and/ or chemical formulations and professional, mechanical removal of plaque and calculus (47).

2.4.1. Supra-gingival Biofilm Control

Supra-gingival biofilm control is essential in the prevention of periodontal diseases. In order to control biofilms supra-gingivally, oral hygiene products in terms of mechanical devices and adjunctive chemical formulations are designed, developed and marketed to provide optimal oral health care (9, 48).

2.4.1.1. Mechanical Biofilm Control

Meticulous supra-gingival mechanical biofilm control has been demonstrated to be an effective means of preventing the initiation and/or progression of the periodontal diseases. Mechanical disruption of the MDB cause physical destruction and elimination of biofilm matrix components and thus reduces the pathogenic microbiata in oral cavity which modifies both quantity and quality of biofilm. In terms of supra-gingival mechanical biofilm control, toothbrushes and interdental cleaning aids are the most commonly used oral hygiene aids, but there are several other devices such as powered toothbrushes, ionic toothbrushes, chewing sticks, chewing sponges, tree twigs, etc. for the removal of MDB (49). It has been shown that the use of either a manual or a powered toothbrush, together with effective inter-dental cleaning devices are the most effective daily mechanical biofilm control regimens (50-53). The American Dental Association (ADA) recommendations for daily oral care regimens are toothbrushing twice a day and flossing once a day as a regimen to obtain optimal oral hygiene levels (54).

However, most patients find it difficult or impossible to comply with the exacting level of plaque removal required to obtain optimal oral health. Reasons for noncompliance are many and may include things such as culture, overall level of education, beliefs and attitudes regarding personal care, frequency of dental visits, age, manual dexterity, inadequate time and frequency of brushing and poor brushing techniques (44). An additional limitation of mechanical plaque control procedures is that they concentrate solely on the hard surfaces of the oral cavity (55). Recent studies have demonstrated that microorganisms involved in the etiology of gingivitis and periodontitis

accumulate on several soft tissue surfaces of the mouth, which serve as a source of bacteria for the colonization of tooth surfaces. There are also some circumstances in which adequate mechanical plaque control is not possible, including after oral or periodontal surgery, in patients with interdental maxillary fixatitions, in acute mucosal or gingival infections where pain preclude mechanical hygiene and in mentally or physically handicapped patients (3).

Above mentioned reasons provide the basis for development of chemical plaque control agents that would augment mechanical plaque removal. As a consequence, the combination of mechanical and chemical oral hygiene offers the greatest efficacy, because the bulk of plaque is reduced mechanically, leaving behind only disorganized and thin dental plaque that can easily be further reduced by chemical means (11). Chemical anti-plaque agents present in different aids could reach these soft tissue surfaces, improving the control of biofilm growth on these surfaces and delaying microbial accumulation on teeth and promote periodontal healing (49).

2.4.1.2. Chemical Biofilm Control

The adjunctive use of chemical biofilm control agents are necessary in those subjects who are unable to properly control supra-gingival biofilm with mechanical devices in order to assist in the prevention of plaque accumulation and occurance of gingivitis (13). These agents present in different aids could reach these soft and hard tissue surfaces, improving the control of biofilm growth on these surfaces and delaying microbial accumulation on teeth and promote periodontal healing.

2.4.1.2.A. Classification of Chemical Biofilm Control Agents

Depending on the antimicrobial efficiency and relative substantitivity, Kornman et al. (56) classified chemical agents as;

- First-generation agents those show very limited substantivity with limited time of action.These agents reduce plaque score by 20-50% and examples for this group of chemicals are antibiotics, phenolic derivatives, plant extracts, fluorides, quaternary ammonium compounds and oxidizing agents.
- Second-genaration agents those show good substantivity with prolonged time of action These agents reduce plaque score by 70-90% and examples for this group of chemicals are bisbiguanides; CHX is the best example.

- Third-generation agents those interfere or prevent bacterial adhesion with no effect on bacteria such as alcohols and delmopinol and also products containing sanguinarine, oxygenating agents, saturated pyrimidine and hexetidine.

According to Mandel et al. (57), the antimicrobial agents were categorized as;

- Antiseptics which demonstrate a broad spectrum of antibacterial activity and kil lor prevent the proliferation of all plaque microorganisms,
- Antibiotics which are capable of inhibiting or killing a specific group of bacteria,
- Enzymes which are capable of diffusing in matrix and modify the plaque activity
- Modifying agents which are non-enzymatic, dispersing, denaturating agents that alters the structure or metabolic activity of plaque bacteria,
- Antiadhesives which interferes with the attachment of bacteria to the pellicle or to each other.

According to their individual properties, Eley et al. (12) classified chemical biofilm control agents as;

- Group A (anti-plaque) agents those having good substantivity which show antibacterial as well as antiplaque action. These agents inhibit plaque formation to such an extend that they prevent development of gingivitis. This property makes these agents preferable to mechanical cleaning methods for a short time of period. Examples for this class of chemical agents include chlorhexidine, acidified sodium chlorate, saliflour and delmopinol.
- Group B (plaque-inhibitory) agents have little or no substantivity but with a good antibacterial spectrum. .Therefore, these agents should be used adjunctive to mechanical plaque control regimens Cetylpyridiniım chloride and triclosan rinses are examples fort his group of chemical agents.
- Group C agents have low to moderate activity on plaque bacteria and should be used as a cosmetic expectations such as breath freshening. Examples for this group of chemical agents are sanguinarine, oxygenating agents, hexetidine and rinses containing the saturated pyrimidine.

According to their effects, Lang and Newman (58) categorised chemical biofilm control agents as;

- Antimicrobial agents; those having bacteriostatic or bactericidal effect in vitro and cannot show its effcacy *in vivo* alone,
- Plaque reducing/inhibitory agents; those having qualitative or quantitative effect on biofilm which may or may not affect occurance of gingivitis and/or caries,

- Anti-plaque agents; those affect the plaque sufficiently to show a benefit in terms of gingivitis control and
- Anti-gingivitis agents; which reduce gingival inflammation without necessarily affecting dental plaque.

These definitions are widely accepted in Europe, but in North America the term "antiplaque" refers more often to agents capable of significantly reducing plaque levels and "antigingivitis" to agents capable of significantly reducing gingivitis levels .

2.4.1.2.B. Mechanism of Action of the Chemical Biofilm Control Agents

Chemical agents act on biofilms by quantitatively those reducing the number of microorganisms and/or qualitatively those altering the vitality of biofilm composition. According to their mechanism of action, these agents categorized as,
- Anti-adhesive agents prevent initial adhesion of microorganisms surface by forming a thin layer over tooth surface which interferes with primary plaque forming bacteria matrix formation. Antimicrobials have either bactericidal effects those inhibiting bacterial proliferetion and co-aggregation or bacteriostatic effects those interfere with bacterial division either attaching or already attached bacteria tot tooth surfaceon biofilm.
- Plaque removal agents disrupt biofilm from tooth surfaces by detachment and/or biofilm elimination through, breaking the chemical links between the tooth surface and the biofilms.
- Anti-pathogenic agents alter the biofilm pathogenicity without necessarily destroying the microorganisms or enhancement of host immune systems by different mechanisms (46).

2.4.1.2.C. Evaluation of Activity of Antiplaque Agents for Supra-gingival Chemical Biofilm Control *In-vivo*

In periodontology, *in-vivo* clinical trials have been used to test the effectiveness of chemical biofilm control agents that inhibit plaque formation, influence plaque removal and prevent or reduce gingivitis and calculus formation. Each one of these studies answer spesific research questions at certain stages of product evaluation.
- Eight-hour substantivity studies are used to test how long a chemical formulation performs a persisting antimicrobial effect in vivo (15). The substantvivity of a chemical formulation depends on the ability of a substance of physical and chemical bonding to a surface as well as its resistance against removal or inactivation, as long as it remains

biologically active. As the first test stage, failure of a formulation to show substantivity would prove an inappropriate effect on the inhibition of plaque development. If there is an effect then according to the obtained efficacy profile an optimal frequency of use for any product can be recommended. The substantivity of an agent determines the rinsing frequency needed, however, practically the rinsing frequency is limited to 2 or 3 times a day. After application of a single rinse on pre-existing plaque, plaque and saliva bacteria are studied for the following eight hours while the participants cease all oral hygiene measures (59).

- Plaque re-growth studies also known as 3 or 4-day non-brushing models, aims to test the plaque inhibitory effect of a formulation *in vivo* while any oral hygiene is stopped during the test phase. Primarily plaque-free teeth undergo a three or four-day period with no oral hygiene measures, except the rinsing with the allocated formulation. A final plaque assessment shows whether or not the mouthrinse *per se* is able to depress plaque development and to what extent. If no plaque inhibition can be shown in this type of study no further effect of the rinsing solution can be expected in studies when oral hygiene is performed. Therefore, this model seems to be the second stage in product testing .
- Experimental gingivitis models have the same design as plaque regrowth models but test the formulation for longer periods of time, typically 12–28 days allowing for the evaluation of gingivitis indices (2). No mechanical hygiene measures are permitted during the test period (60).
- Home use studies; are long-term studies to test the efficacy of anti-plaque and anti-gingivitis agents under almost real-life circumstances. This model refers to the FDA requirements that ask for safety records for oral hygiene products as well. The study was performed in parallel groups. In addition to the rinsings, mechanical oral hygiene was part of the protocol (61).

2.4.1.2.D. Delivery Formats for Chemical Plaque Control Agents

Chemical agents are designed in different delivery formats such as mouthwashes, gels, dentrifices, chewing gums, aerosols or spreys, varnishes, sustained release devices, lozanges and irrigators.

Mouthwashes are medicated, non-sterile aqueus solutions used for gargling and rinsing the mouth generally classified as either cosmetic or therapeutic or a combination of these (62). The use of mouthwash to control plaque bacteria date back around 5000 years when the Chinese recommended the use of child’s urine for the control of gingivitis

(63). A major advantage of a mouthwash is that they allow access to difficult -to-reach areas, easy to use and well accepted by patients with a pleasant taste (64).

Mouthwashes can be used in various clinical conditions such as; an adjunct to mechanical oral hygiene procedure in conditions like: after subgingival scaling or root planing, in patients having inadequate oral hygiene and post-scaling cervical hypersensitivity. Mouthwashes can be used for various preventative and therapeutic purposes to treat oral infections, reduce inflammation, decrease halitosis and to deliver fluoride locally for preventing caries.

There are two types of mouthwashes according to their indications. Cosmetic mouthwashes are over the counter products and used to supress bad breath and refresh mouth with a pleasent taste. Therapeutic mouthwashes are available both over-the-counter and by prescription, and used as an antimicrobial, a topical anti-inflammatory agent, a topical analgesic or for caries prevention depending on the formulation. Also they can be used to replace normal toothbrushing which is not possible in various conditions like: after periodontal surgical procedures, after intermaxillary fixation, during acute oral or gingival infection, for mentally or physically handicapped patients (65).

Molecules included in the commercial mouthwashes are derived from anticeptic, disinfectant and preservation research areas and usually consist of a mixture of the active chemical agent, water and ethanol as a solvent, surfactants, humectants, flavoring agent, sweeteners, coloring agents and preservatives (66).

Consequently, when producing a brief for a formulator of antiplaque mouthrinses, essential elements must include;

- The use of antibacterial agents with ; antiplaque properties demonstrated in long-term clinical trials and proven safety at effective dose levels for the intended period of use (e.g. chlorhexidine, essential oils combination, CPC, triclosan etc);
- Mixing with at least 50% of water;
- Adding solubilizers for non-water soluble ingredients, that is, ethanol or emulsifiers such as surfactants. making the solution palatable and likeable (add flavour oils – sweeteners, ethanol, colourants, etc.) (67).
- Making it stable, mainly by preventing precipitation (add stabilizers such as surfactants, solubilizers such as ethanol, pH adjusters, etc.).
- Making it stay stable for shelf-life (add preservatives such as antibacterials and antifungals, e.g. ethanol etc.).

2.5. Chemical Anti-plaque Agents Formulated in Mouthwashes

Anti-plaque agents are defined as chemicals which have an effect on plaque sufficient to benefit gingivitis and/or caries (68).

In 1985 the Council on Dental Therapeutics of the ADA was established guidelines for the acceptance of anti-plaque/gingivitis agents (56). According to these guidelines, a chemical agent could prevent or reverse gingivitis if it:

(i) eliminates all plaque; or

(ii) reduces plaque below an individual's threshold for disease; or

(iii) alters the bacteria of plaque in such a way that health would not convert to disease.

These agents could also have a mechanism of action to (45),

a) interfere with the adhesion of oral bacteria to surfaces and prevent biofilm formation,

b) interfere with co-aggregation mechanisms or to affect bacterial vitality which thereby prevent further growth of colonies, or

c) remove or to disrupt existing biofilms.

In addition, it has been suggested that chemical agents could also affect gingivitis directly if they possessed anti-inflammatory activity (69).

Enzymes are categorized into two groups according to their mechanism of action on plaque biofilm.The first group including dextranases, mutanases, proteases and lipases act on biofilm structure by interfering with bacterial attachment or disintegrating existing plaque on tooth surfaces. The second group including glucose oxidase and aminoglucosidase act by enhancing host defence mechanisms by salivary lactoperoxidase system which conver thiocyanate to hypothiocyanite. No long term studies are available and results of the studies in vivo on gingivitis are contradictory (70).

Bisbiguanides; CHX, alexidine, and octenidine, show their antiplaque activity by binding to cell membranes and have the ability to kill a wide range of microorganisms by damaging the bacterial cell wall. CHX is considered as a gold standart antiplaque and antigingivitis agent act by increasing the permeability of cell membrane followed by coagulation of cellular macromolecules. It also is effective against both Gram- positive and Gram-negative bacteria including aerobes and anaerobes, yeasts, fungi and lipid enveloped viruses. It increases the permeability of cell membrane followed by coagulation of cellular macromolecules.

Quaternary ammonium compounds, benzylyconium chloride and cetylpyridinium chloride (CPC), are monocationic surface-active agents. CPC act by reducing surface tension, adsorbing to negatively charged surfaces and disrupting bacterial cell membranes

causing leakage of intracellular components. It shows moderate plaque inhibitory activity as compare to CHX because of its rapid desorption from the oral mucosa and its monocationic nature. The single cationic group binds to mucosa providing mucosal retention but leaving few unattached sites for its antibacterial action.

Essential Oils (EO), phenolic compounds, are a fixed blend of thymol, eucalyptol, methyly salicylate, benzoic acid and boric acid and menthol in an alcohol solvent. They are broad spectrum antimicrobial agents those decrease bacterial multiplication, aggregation and pathogenicity causing destruction of bacterial cell and inhibition of bacterial enzymes. They also have anti-inflammatory activity, prostaglandin inhibitory activity and antioxidants activity (71). Listerine , is a combination of the two phenol-related essential oils, thymol and eucalyptol, mixed with menthol and methylsalicylate in a hydroalcoholic vehicle. The agent is used in a mouthrinse form. The effects of this agent on plaque growth and gingivitis are well documented both short and long-term. Drisco et al showed that, EO-containing mouthwash is effective in reducing plaque and gingivitis in interproximal areas, and is as effective as floss in reducing interproximal plaque and gingivitis (72). They can be recommended as an adjunct to mechanical plaque control measures especially in patients with gingival inflammation even with regular tooth brushing and flossing.

Oxygenating agents, Hydrogen peroxide, Sodium peroxyborate and peroxycarbonate are well known because of their use in cases of acute necrotizing ulcerative gingivitis and pericoronitis. These agents are broad spectrum antimicrobial bleaching agents having strong oxidising properties, act by releasing nascent oxygen to loosen debris, remove stains and kill anaerobic micro-organisms Short-term studies demonstrated the efficacy of hydrogen peroxide alone in reducing plaque and gingivitis (73). The combination of 5% povidone–iodine and 1.5% hydrogen peroxide in a rinse formu- lation has also shown usefulness against plaque and gingivitis (74). Several studies indicate that the use of oxidizing mouthwashes containing peroxyborate or hydrogen peroxide may help control the dental stain associated with CHX use (75). They are recommended for acute ulcerative conditions, to relieve soreness caused by dentures, orthodontic appliances and for stain removal.

Amine alcohols, delmopinol and octapinol, are surface active agents which has limited antimicrobial activity in vitro and in vivo studies. The suggested mechanism of action od delmopinol is its interference with plaque matrix formation and reduction of bacterial adherence which would cause the plaque to be more loosely adherent to the tooth

so that it would be more easily removed by mechanical cleaning procedures, and would therefore be suitable for a pre-brush mouthrinse (76). Reported adverse effects such as dental staining,feeling of mucosal numbness and burning sensation limits their usage. Studies on delmopinol focused on its anti-plaque activity; when compared to CHX, delmopinol was found to be less effective in terms of antimicrobial activity but more tolerable and with less adverse effects (61).

Detergents are common ingredients in toothpastes and mouthwashes, which has foaming and surfactant activity that reduces the surgface tension and creates the impression of cleanliness. Sodium lauryl sulfate (SLS) is the most frequently used detergent with a limited antimicrobial and antiplaque effect. SLS interacts with components of the bacterial cell membrane and adsorbs and penetrates through the cell wall, thus results in leakage of intracellular components and cause cell lysis.It has a limited usege only in dentrifices because it eliminates protective mucin layer from the mucosa and cause adverse effects such as cheilitis, stomatitis, burning sensation and desquamation.

Triclosan, is a non-ionic antiseptic compound which has both anti-bacterial and anti-inflammatory properties. The antibacterial action seems to be associated with the cytoplasmic membrane disruption of the bacterial cell by the prevention of the amino acid uptake, whereas its anti-inflammatory action lies on the inhibition of the oxygenase/lipoxyge- nase pathway in the arachidonic acid metabolism. It has been used as in dentifrice or mouthrinse formulations. The safety of several triclosan-containing formulations has been established by several long-term studies with no shifts in the microflora of the supragingival plaque and no immergence of opportunistic pathogens (77). Various studies have shown that Triclosan reduces the inflammatory reaction on the gingiva by sodium lauryl sulphate and reduce the severity and healing period of recurrent apthous ulcers. Gaffar et al stated Triclosan reduces the levels of inflammatory mediators (prostaglandins and leukotrienes) by inhibiting both cyclo-oxygenase and lipoxygenase pathways. Triclosan also increases the binding ability of mouthwashes to the oral mucosa and thus being available for a longer period of time.

Povidone-iodine is a broad spectrum antimicrobial agent which has affinity against bacteria, virus, fungi and protozoa. It is an iodophore in which iodine is loosely bound to Povidone thereby delivering free iodine to bacterial cell membrane. It reduces plaque formation and decreases the severity of gingivitis and radiation mucositis. It is contraindicated in individuals having sensitivity to iodine and pre-existing thyroid

disorders.

Natural products; plant, fungal, microorganism, animal, and marine extracts have been used in oral hygiene products for many years and had shown an important growth demand from the markets and professional community. They possess antiinflammatory and anti-oxidant activities that are beneficial to oral health and act by reducing both bacterial adhesion to tooth and restorative materials surfaces and the oxidative burst from neutrophils. Sanguinarine, chamomile, echinacea, sage, clove, myrrh, rhatany, peppermint oil, tea tree oil, meswak, aloe vera, turmeric, neem, green tea, propolis, xylitol and hyaluronan had shown an important growth demand from the markets and professional community.

Xylitol; is a natural nonfermentable five-carbon alcohol derived from fruits, vegetables and berries. It is artificially isolated from xylan-rich plant materials and it has been widely researched and globally accepted as a natural sweetener approved by the US Food and Drug Administration. It is formulated in chewing gums, gummy bear snacks, syrups, dentrifice and mouthwashes. Xylitol reduces the levels of *mutans streptococci* in plaque and saliva by disrupting their energy production processes, leading to futile energy cycle and cell death. It reduces the adhesion of these microorganisms to the teeth surface and also reduces their acid production potential. The effect of a combination of xylitol and chlorhexidine on the viability of *S. sanguis* or *S. mutans* during the early stages of biofilm development has been studied in comparison with xylitol and chlorhexidine alone (78). This study showed that the xylitol/chlorhexidine combination inhibited streptococci more when compared with xylitol or chlorhexidine being used alone (78). This newly discovered synergistic action could be used for high-risk caries patients or for reducing *mutans streptococci* transmission from mother to child. Chlorhexidine alone and xylitol/chlorhexidine solutions are effective against both *S. mutans* and *S. sanguis*. *S. sanguis* was most sensitive to the antiseptic effects of chlorhexidine alone, while *S. mutans* colonies were more sensitive to the xylitol/chlorhexidine solution (79).

Hyaluronic acid (HA), also known as Hyaluronan, a naturally occuring polysaccaride, is the main component of the extracellular matrix of many tissues, organs and fluids in humans (80). HA has been identified in all periodontal tissues, as in non-mineralized tissues (gingiva and periodontal ligament) and in mineralized tissues (cementum and alveolar bone). In addition, high levels of hyaluronan are present in circulating blood serum and identified in nearly all GCF samples. It is also found in the glycocalyx of some strains of bacteria such as *Streptococci,* where it can act as a host

defence mechanism (81). HA has many structural and physiological functions within tissues such as; extracellular and cellular interactions, growth factor interaction and regulation of osmotic pressure and tissue lubrication . HA possesses an extremely high capacity to bind water which produces an anti-oedematous effect (82). It combats the inflammation caused by hyaluronidase-producing bacteria by inactivating the enzyme.

HA regulates cell permeability and reduces abnormally high capillary permeability. This helps to prevent infestation by infectious micro-organisms, thus inhibiting tissue destruction . HA has been proposed as an adjuvant in the treatment of gingivitis (20). Among its various properties, several studies have recently shown the ability of HA to protect against various infectious agents, depending on HA concentration and molecular weight, while more recently HA interference on bacterial adhesion and biofilm formation has been extensively investigated (83).

Table 2.1. Chemical Anti-plaque Agents Formulated in Mouthwashes (68).

COMPOUNDS	AGENTS
Enzymes	Protease Lipase Nuclease Dextranase Mutanase Glucoseoxidase Amyloglucosidase
Bisbiguanides	Chlorhexidine Alexidene Octenidine
Quaternary Ammonium Compounds	Cetyl pyridinium chloride Benzalconium Chloride
Phenolic compounds, Essential Oils	Thymol 4-Hexylresorcinol 2-Phenylphenol Eucalyptol Listerene
Fluorides	Sodium fluoride Sodium monofluorophosphate Stannous fluoride Amine fluoride
Metallic ions	Copper Zinc Tin
Oxygenating agents	Peroxides
Natural Products	Herbal products Xylitol Hyaluronic acid
Other Antiseptics	Iodine Povidone iodine Chloramine-T Sodium hypochlorite Hexetidine Triclosan Salifluor Amine alcohols

2.6. Chlorhexidine

Chlorhexidine (CHX) is a cationic anticeptic compound used for chemical biofilm control and prevent gingivitis. It was first discovered during antimalarial drug researchs at the end of 1940's by The Imperial Chemical Industries Limited. They synthesized a group of compounds known collectively known as the polybiguanides that demonstrated a broad spectrum of antimicrobial activity. Modifications to the chemical formulae and further explorations of the chemical structure of the polybiguanides led in the 1950s to the synthesis of the bisbiguanides, and finally they synthesized the compound with the highest bacteriostatic and bactericidal effects. That compound became known as CHX and still considered as the gold standart anti-plaque agent (84).

CHX is a symmetrical molecule consisting of four chlorophenyl rings and two biguanide groups connected by a central hexamethylene bridge. CHX is a strong base and is bi-cationic at pH levels above 3.5., with the two positive charges on each side of the hexamethylene bridge (85). One charged arm of CHX structure binds to the tooth surface, whereas the other arm interact with the bacterial membrane and that interaction is recognized as a pin cushion effect. It reversibly and tightly binds to tooth surface, oral tissues and dental plaque bacteria and releases slowly over time resulting in 8-12 hours of sustained antimicrobial activity. CHX's superior activity is attributed to its high subtantivity and pin-cushion effect (86).

The most important *in vivo* study published by Löe and Schiott in 1970, revealed that CHX is a highly effective anti-plaque agent. The study showed that two daily rinses with 10 mL of 0.2% CHX-containing mouthrinse prevented plaque formation and gingivitis development in the absence of normal mechanical tooth cleaning. Further, they found that CHX continued to prevent plaque and gingivitis in the oral cavity up to 24 hours after use (14).

2.6.1. Mechanism of Action of Chlorhexidine

The primary mechanism of action of CHX involves membrane disruption, causing concentration-dependent growth inhibition and bacterial cell death. Bacterial cell membranes contain phosphate groups and have a net negative charge. The positively charged CHX molecule is electrostatically attracted to negatively charged bacterial surfaces. Adsorption of CHX to the outer membrane increases the permeability of the bacterial cell membrane, and cause leakage of small molecules such as potassium. As a result of this interaction, physical integrity of bacterial cell membrane is damaged. At this

stage, the effect of CHX is bacteriostatic and reversible and exert this function at low concentrations. At higher concentrations, CHX precipitates cytoplasmic proteins in bacterial cell membrane and the effect become bacteriocidal to organisms exposed to it. This stage is irreversible and lethal to the cell. The actual concentrations at which the effect is bacteriostatic or bactericidal varies according to the bacterial species under investigations (87).

CHX is maintained in the oral cavity after having been adsorbed onto the tooth surface and oral mucosal surfaces. The dicationic nature of CHX contributes significantly to its substantivity. Substantivity is defined as the ability of chlorhexidine to remain effective in inhibiting plaque for an extended period of time, contributes to its efficacy. The substantivitiy is effected by intrinsic and extrinsic factors. Intrinsic factors such as concentration, time of application, and temperature can affect the retention of chlorhexidine in the oral cavity. Concentration, volume, and duration were varied to investigate these intrinsic factors *in vivo*. Results showed the volume of mouthrinse did not affect substantivity although concentration and duration did. Substantivity was increased with higher concentrations and longer treatment time. Eating, drinking water, chewing sugar-free gum, and smoking a cigarette were extrinsic factors investigated. It was found that the substantivity of 0.2% CHX decreased significantly with these activities. These findings highlight the importances of dietary etiological factors.

The proposed mechanisms of action of CHX which prevent bacteria from colonizing on teeth by;

i) inhibiting the formation of the acquired pellicle by binding to the acidic groups of salivary glycoproteins,

ii) adsorbing to the extracellular polysaccharides of the tooth in the acquired pellicle, or

iii) competing with calcium ion agglutination factors in plaque.

2.6.2. Usage of Chlorhexidine in the Field of Dentistry

CHX products are available in many forms such as mouthrinses, gels, spreys, toothpastes, varnishes and chewing gums. And also a number of commercially prepared CHX mouthrinses are available at concentrations of 0.2%, 0.1%, 0.12%, 0.03%, 0.05%, 0.06%. Based on the clinical situation, the duration of the product usage and the main objective of the intervention, CHX have been proposed for;

- Single usage as a preoperative irrigation rinse to reduce bacteremia, bacterial load in oral cavity and aerosol contamination associated with sonic and ultrasonic devices (88),
- Short-term usage for the prevention of biofilm formation as adjunct to mechanical plaque control regimens in the treatment of gingivitis and periodontitis, acute mucosal infections and also in patients with inter-maxillary fixatitions.or as sole use where mechanical plaque control aids can not be used after periodontal and implant surgeries to prevent postsurgical infections.
-Long-term usage of CHX can be indicated in order to prevent biofilm formation which mechanical plaque control is impossible.

CHX is also used in disinfection of dental prosthetics and orthodontic appliances Disinfecting complete or partial dentures by immersing them in 0.2 % CHX solution at night can decrease the incidence of denture stomatitis (89). Recently, the postoperative use of CHX mouth rinses has replaced periodontal packs as the standard periodontal surgical care used to enhance healing in an infection-free environment (90). Other periodontal applications of CHX include its adjunctive use in total mouth disinfection, and as a substitute for saline in cooling ultrasonic tips (91). Patients with inter-maxillary fixation and those who are mentally challenged will benefit from the antimicrobial effects of CHX as a substitute for and adjunct to mechanical plaque control. The incidence and duration of minor (92) are reportedly decreased following CHX use. Other reported uses of CHX include using it as a root canal disinfectant, for the treatment of halitosis and as disinfectant prior to performing oral surgical procedures (93, 94).

2.6.3. Toxicity and Adverse Effects of Chlorhexidine

Systemic absorption of CHX by the mucosa of the gastrointestinal tract is virtually non- existent and appropriate use of CHX is generally considered to be safe and non-toxic. Animal experiments with radiolabelled CHX have shown that the half-life of CHX is 4 daysand the primary route of excretion is through the feces with minimal metabolic changes. Systemic toxicity, microbial resistance and superinfection don not ocur with the oral use of CHX (95). But, there are many local adverse effects that have been reported including the brownish staining of the teeth and dorsum of the tongue, taste disturbances, particularly, epithelial desquamation, soft tissue lacerations, parotid salivary gland swelling and increased supra-gingival calculus formation (9).

2.7. Hyaluronan

Hyaluronan, also known as hyaluronic acid (HA), is a negatively charged and non-sulphated linear GAG found naturally in vertebrate organs, fluids, connective tissue, epithelial and neural tissues. In humans, HA is abundant in the vitreous of the eye, the umbilical cord, synovial fluid, heart valves, skin, and skeletal tissues (16). It is a ubiquitous component of the vertebrate ECM and participates in a wide variety of physicochemical and biological processes (16).

HA was first isolated from the vitreous body of bovines' eyes. They named the "hyaluronic acid" from the sum of the words hyaloid (vitreous, which means glass in Greek) and uronic acid. The chemical structure of HA is composed of D- glucuronic acid and N- acetylglucosamine (1:1) linked together through alternating beta-1, 4 and beta-1, 3 glycosidic bonds (16).

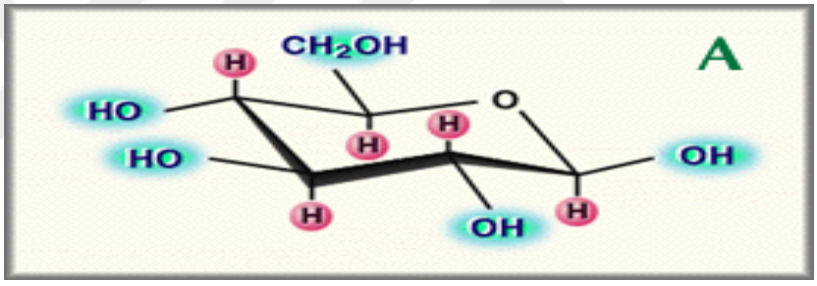

Figure 2.3. Chemical structure of Hyaluronan as a Disaccharide (16).

In physiological solution, the mainstay of a HA polymer structure is reinforced by a combination of the chemical structure of the disaccharide, internal hydrogen bonding of adjacent sugar units, and interactions with solvent. The axial hydrogen atoms form a non-polar, relatively hydrophobic face while the equatorial side chains form a more polar, hydrophilic face, thereby creating a twisting ribbon structure. Thus, HA molecule presumes an expanded random coil structure in physiological solutions, which have the ability to bind large amounts of water (16, 96). One gr of HA can hold up to 6 L of water.

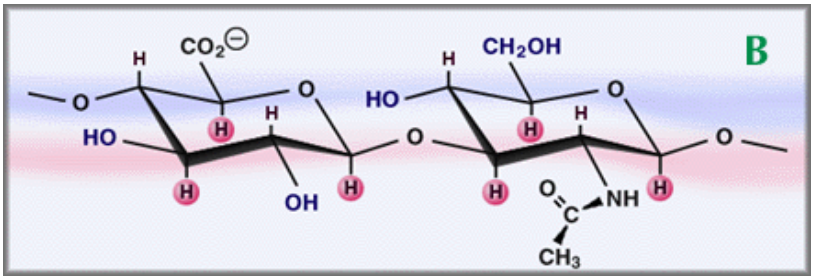

Figure 2.4. Polymer Structure of Hyaluronan as a Polysaccharide (16).

HA is synthesized on the inner surface of the cellular plasma membrane by HA synthase (HAS) enzymes and transported out of the cell to the extracellular space with lengthening of the polymeric chain. Three mammalian HAS genes (HAS1, HAS2, HAS3) have been characterized and responsible for the synthesis of high molecular weight HA (HMW-HA). Each gene differs in their tissue specificity and in the general size of molecules they produce. HMW-HA is degraded into low molecular weight-HA (LMW-HA) fragments by hyalurinidase enzymes in the extracellular space, by some proteases and by the action of reactive oxygen species in the injured tissues. Degradation of HA is as important as synthesis because, HA have different biological activities depending on polymer size. The turnover of HA is a rapid process, as the half life of HA molecule in the bloodstream is only about 2-5 minutes (97).

2.7.1. Properties of Hyaluronan

HA molecule can take different forms, as the acid form named Hyaluronic acid, and the salt form named sodium hyaluronate, which forms under physiological conditions (pH 7.0) (98). It is highly negatively charged that can absorb large amounts of water and expand up to 1000 times forming a loose hydrated network which functions as a space filling material, lubricant and osmotic buffer in the native ECM (96). The hydrated HA network acts as a filter responsible for the transport of water and depriving the movement of pathogens, plasma proteins and proteases. HA is also responsible for the regulation of tissue repair and disease processes during injury (99), by activation of inflammatory cells to trigger an innate response to injury and by regulation of behavior of epithelial cells and fibroblasts (100) (82, 97). HA is recognized by cell surface receptors. Interaction of HA with its receptors on cells, several intracellular signaling pathways are triggered, which in turn regulate adhesion, proliferation, migration and differentiation of cells (17, 80).

HA is an essential component of intact, healthy gingiva and oral mucosal tissue

(101). It has been identified in all periodontal tissues, apparently in the non-mineralized tissues such as gingiva and periodontal ligament and low quantities in mineralized tissues such as cementum and alveolar bone. It tends to concentrate particularly in those superficial layers of the gingival epithelium, where it acts as a barrier, thus supporting stability and elasticity to the underlying periodontal connective tissues. It is synthesized by hyaluronan synthase enzymes in various cells from the periodontal tissues, including fibroblasts and keratinocytes in gingiva and periodontal ligament, cementoblasts in cementum and osteoblasts in alveolar bone (97).

2.7.2. Mechanism of Action of Hyaluronan

HA is present in the extracellular matrix, on the cell surface, and inside the cell. Functions of HA differ where it presents, and these are classified as associated with the organization of the extracellular matrix, associated with a formation of a HA coat on the cell surface and associated with receptor-mediated signaling.

HA in extracellular matrix regulates water retention and homeostasis through its viscoelastic and hygroscopic nature. In the hydrated state, much of the water around the HA molecule is immobilized and thus energetically very stable. This results in restriction of movement of water and small molecules in extracellular matrix. It exhibits a high resistance against water flow and thus acts as a barrier, lubricator and shock absorption in tissues. This property also inhibits penetration of viruses and bacteria into the periodontal tissues, thus prevents tissues from bacterial invasion. HA acts as a sieve and allows small molecules move freely while larger particles are immobilised. Also it has been proposed that hyaluronan and other polysaccharides regulate transport of other macromolecules through the extracellular space .

HA is well known as a binding agent between various connective tissue components. It also has various biological functions that include an important role in cell adhesion, migration and differentiation, enhancement of tissue regeneration, stimulation of osteoinduction, involvement in the process of osseointegration (102) and plays an important role in the early stages of wound healing (103). It has adhesive properties and also possesses anti- inflammatory properties (20). It is shown to have both a bacteriostatic effect and antioxidant effect.

HA has bone induction characteristics with osteogenic substrates such as bone morphogenic protein, calcitonine gene- related peptide and osteopontin. It accelerates the bone regeneration by means of proliferation, chemotaxis and successive differentiation

of mesenchymal cells. Recent studies also demonstrated that HA aids in the repair process of both soft and hard tissue. Hence it has been suggested that HA could be used for bone regeneration in periodontal/peri-implant diseases.

HMW-HA reduces cell proliferation in fibroblasts and lymphocytes as well as in epithelial cells which abates the inflammatory process. This property is useful in improving the periodontal lesion as in patients with chronic periodontitis.

Hyaluronidase is a bacterial enzyme which plays an important role in plaque induced‟ diseases due to its ability to break down the proteoglycan and GAGs in the ground substance of connective tissue (ie. macroaggregates of hyaluronic acid and proteins), thereby enabling bacteria to invade even the deepest periodontal structures. As a result of its physiological macroaggregating activity high molecular weight hyaluronic acid can therefore perform an efficient anti- hyaluronidase action.

Oedema basically consists of an accumulation of fluid in the intercellular spaces of connective tissue. In the proteoglycans, the macroaggregating effect of hyaluronic acid gives rise to “free water binding”. Therefore capture of free water resulting from the formation of hydrogen bonds produces an anti oedematous effect helping in tailing the inflammation.

Regardless of the concentration or molecular weight HA has no bactericidal effect but it exhibits bacteriostatic effect. Significant bacteriostatic effects were observed regardless of the concentration or molecular weight for S. Aureus and to a greater extent for A. Actinomycetencomitans. The most significant bacteriostatic effects on both these strains were observed with high concentrations of medium molecular weight Hyaluronic acid. It has least bacteriostatic effect on S. Mutans and P. Gingivalis strains.

High concentration of HA has been demonstrated in tissue repair. It is believed to play a role in wound healing by facilitating cell migration and differentiation during embryonic development and tissue repair. Numerous proteins have been shown to bind HA, including fibrinogen, fibrin, fibronectin and collagen. All these molecules help in wound healing and are also present even in foetal wound matrix. With the increased concentration of hyaluronan can modulate foetal wound healing by orchestrating healing through regeneration rather than scarring

It stimulates the neutrophils and macrophages by enhancing their phagocytic activity, and further stimulates the release of chemotactic factors for the fibroblasts and helps in fibrin development. Moreover, it induces the fibroblasts proliferation and stimulates their metabolism during the granulation phase of the healing process, with a

subsequent increase in collagenous fibers and in fundamental substance deposition.

It is a glycosaminoglycan with anti inflammatory effect. Its anti-inflammatory effect may be due to the action of exogenous hyaluronan as a scavenger by draining prostaglandins, metalloproteinases and other bio-active molecules (104).

2.7.3. Hyaluronan-Based Biomaterials

The application of exogenous HA and HA based biomaterials has been successful in manipulating and accelerating wound healing by inducing early granulation tissue formation, inhibiting inflammation, promoting epithelial turnover and also connective tissue angiogenesis (17, 105). It has currently been introduced as a drug delivery agent for different routes such as nasal, pulmonary, ophthalmic, topical and parenteral (106, 107).

2.7.4. Usage of Hyaluronan-based Biomaterials in Periodotontology

HA has been used in periodontology as an adjunct to scaling and root planning, as an antimicrobial agent when topically applied to subgingival area, as a bone regenerating agent in periodontal intrabony defects, in guided bone regeneration, and as a graft for gingival augmentation in mucogingival surgeries (20, 80, 108-110). HA is a recent addition to the local chemotherapeutic agents which has shown a number of clinical therapeutic properties.

2.7.5. Studies on Antiplaque Efficacy of Hyaluronan-based Biomaterials

The literature on the effectiveness of HA in the form on mouthrinse on periodontal outcomes is very limited. Rodrigues et al., found HA-containing mouthrinses to be effective in reducing the growth of periodontal pathogens (*Aggregatibacter actinomycetemcomitans* and *Prevotella intermedia*) *in vitro* and had plaque inhibition potential similar to CHX *in vivo*. This single-blinded, parallel design, randomised controlled trial was carried out and the 4-day plaque re-growth model was used to study the efficacy of the three mouthwashes: 0.025% HA-containing mouthwash in comparison with 0.2% CHX and a water-based mouthwash and also to evaluate its antibacterial efficacy on isolated strains of periodontopathogens. Microbiological and clinical evaluation was performed by culturing and using dental indices. Effects of the three mouthwashes were tested on the growth of isolated strains of *Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans* (111) and *Prevotella intermedia.* Results

showed that, in vitro, hyaluronan had a distinct effect on the growth of *Aggregatibacter actinomycetemcomitans* and *Prevotella intermedia* with no effect on the growth of *Porphyromonas gingivalis*. In vivo, the differences between the individual rinse solutions and the water-based solution showed significantly less plaque re- growth with respect to both CHX (P = 0.033) and HA (P = 0.045) when compared to the negative control. The difference between CHX and HA was not statistically significant (P= 0.69). As a conclusion, 0.025% HA-containing mouthwash was comparable to 0.2% CHX-containing mouthwash in inhibiting plaque growth *in vivo*, and it significantly reduced the growth of *Aggregatibacter actinomycetemcomitans* and *Prevotella intermedia in vitro* (112).

Al-bayati et al. (2010) tested the antibacterial effects Oradex, Gengigel and Salviathymol-n mouthwashes using experimental microorganisms included *Fusobacterium nucleatum*, *Streptococcus mitis*, *Streptococcus constellatus*, *Eikenella corrodens.* Antibacterial effect was assessed by diffusion test. Minimum inhibitory cocncentration (113) and assesment of bacterial mortphology was evaluated by scanning electron microscopy (SEM). Results of the study showed that Oradex exhibited a higher antibacterial effect compared to Salviathymol-n and Gengigel. Gengigel mouthwash had a week antibacterial effect against the tested microorganisms. SEM anlaysis showed that CHX made obvious changes in most of the bacteria loss their original shape and became irregular. Salviothymol-n exhibited some significant changes on the cell morphology of the tested species while gengigel failed. The answers concluded that only Oradex and Salviothymol-n can be prescribed as antibacterial mouthwash for the chemical plaque control due to their antibacterial effect (114).

In another study by Al-Bayaty F. et al. antibacterial effects of CHX gel and HA gel were evaluated on dental biofilm. Pooled supra and subgingival dental biofilm were obtained from helathy individuals and incubated both aerobically and anaerobically. *Streptococcus mitis, Streptococcus constellatus, Eikenella corredens and Fusobacterium nucleatum* were selected to represent dental plaque bacteria. Screening of antibacterial activity was performed by the disk diffusion test. MIC test was done to assess the antimicrobial efficiency af Gengigel and CHX gel. Bacterial morphology was assessed by SEM at 3500x, 1000x and 20000x magnifications. Positive results were obtained by disc diffusion test and both gels showed antibacterial activity on the tested microorganisms. CHX gel produced large dimeter inhibition zone while hyaluronan gel produced smaller diameter inhibition zone. The MIC value of CHX gel for

Staphylococcus auresus, Streptococcus mitis and Streptococcus. constellatus was found 0.2%. However the MIC value of Gengigel could not be detected. The authors declared that CHX gel demonstrated stronger and obvious antibacterial characteristic when compared to hyaluronan containing gel .

Vishal N. et al. evaluated the effect of HA gel formulation in the treatment of plaque induced gingivitis patients both clinically and microbiologically. It was designed as longitudinal, randomized and placebo controlled clinical trial. 105 patients were included. Patients were instructed to apply hyaluronic acid gel in addition to their oral hygiene regimens. The clinical parameters including PI evaluated by Turesky Modification of QHPI-s, GI assessed by Sillness Löe Gingival Index, Papillary Bleeding Index (PBI) were assessed at 1 week, 2 week and 4 weeks intervals. All patients were evaluated based on demoghraphical and clinical data at baseline and divided into 3 groups (35 for each) randomly.

- Negative control: only scaling
- Placebo control: scaling+ placebo gel
- Test group: Scaling+ Hyaluronic acid

Samples for microbiological assessment were obtained just before starting therapy and monitored at the interval of 4 weeks from baseline and evaluated by culture method. Clinical improvements were seen in all clinical parameters. However the comparison of the changes of clinical parameters between groups, statistically significant differences were detected in favour of HA group. However, no significant differences were observed in pair wise comparison in between the groups. Authors concluded that local application of 0.2% HA gel as an adjunct to scaling provided a significant improvement in plaque induced gingivitis however it failed to provide the same improvement in microbiological improvements.

Jenstch et al. assessed the anti-inflammatory and antiedematous properties of HA gel on the treatment of plaque induced gingivitis by a randomized double-blind study. 50 male subjects were divided into two groups; HA gel (0.2%) vs. placebo group . Both groups were instructed to use twice a day and both gels had the same physical consistency, equal colors and taste. No restriction of the individual oral hygiene procedures was given. The clinical readings including PI and approximal PI was assessed.

A randomized, controlled double-blind parallel trial was conducted to evaluate the effects of Gengigel and placebo under controlled conditions in patients with marginal gingivitis when used as a complement to daily oral hygiene over a 4-week period

following a professional oral hygiene session. In each of the 28 gingivitis patients, the four quadrants were subjected to different treatments: scaling, scaling + topical hyaluronan gel, only topical HA gel, and topical + intrasulcular hyaluronan gel. Clinical parameters were recorded at baseline, and on days 7, 14, and 21. Results showed a significant reduction in clinical parameters, inflammatory infiltrates, within the groups. The effect of topical + intrasulcular gel was equivalent to scaling ($P > 0.05$). Topical + intrasulcular HA gel application demonstrated a better reduction than topical hyaluronan gel alone. This study concluded that Hyaluronan gel is an effective topical agent for treating gingivitis, along with scaling and intrasulcular application (115).

A randomized double blind study was evaluated to test gel formulation of HA's effect in the treatment of plaque-induced gingivitis. 50 male subjects with plaque-induced gingivitis were divided into two groups and used a verum or placebo gel twice daily additionally to oral hygiene for a 3-week treatment period. Clinical indices (API, Turesky index, PBI) and crevicular fluid variables (peroxidase, lyso- zyme) were determined at baseline and after 4, 7, 14 and 21days, respectively. Results showed that significant improvements could be found for all clinical variables in both groups. The verum group showed significant improvement in the study area for the plaque indices beginning with day 4 ($p<0.011$) and the PBI beginning with day7 ($P<0.001$) in comparison with the placebo group. The crevicular fluid variables were significantly improved in the centre of the studied inflammation area in the verum group. Here all studied sites had significant decreases in peroxidase (176.72–128.75 and 188.74–128.75U/L) and lysozyme (1.27–0.27 and 1.30–0.33mg/L) activities after 7, 14 and 21 days (*P* between 0.034 and 0.001), whereas in the placebo group only one site showed a significant decrease for lysozyme (1.74–0.75mg/L) after 7 and 21 days (*P*Ω0.048 and 0.025). These data suggest that a HA-containing gel has a beneficial effect in the treatment of gingivitis.

A longitudinal, randomized, parallel and placebo-controlled clinical trial was conducted to investigate the effectiveness of hyaluronic acid gel on patients with chronic plaque induce gingivitis. 105 patients were randomly divided into three groups; negative control group, placebo control group and test group. Patients were instructed to apply gel on inflamed gingiva twice daily in addition with routine oral hygiene maintenance. The clinical parameters Plaque Index (PI), Gingival Index(GI) and Papilla Bleeding Index (PBI) were determined at intervals of 1 week, 2 weeks and 4 weeks from baseline, microbiological parameters were monitored at the interval of 4 weeks from baseline.

Results showed that an improvement of all clinical variables was observed ($p<0.05$) for all treatment modalities. Clinically, there is significant difference ($p<0.05$) for GI & PBI in test group as compared to other groups, but reduction in PI was non-significant. In negative control and placebo control groups, the difference between clinical parameters was non-significant. Statistically significant ($p<0.05$) reduction in percentage of anaerobic Gram negative bacilli and relative increase of Gram positive coccoid cells was seen in all treatment groups at 4 weeks as compared to baseline. A study concluded that, local application of 0.2 % HA gel adjunct to non surgical periodontal treatment provided a significant improvement in clinical parameters than placebo control and negative control groups. Experimental group does not showed any statistically significant results microbiologically (116).

Dahiya and Kamal recently reviewed the biological properties and clinical applications of HA, reporting its positive effect on periodontal disease, and its active role in periodontal wound healing (117).

De Araujo Nobre et al. compared the effects of HA and CHX after the insertion of endosseous implants in two groups of edentulous patients. At short-term follow-up (2 months after sur- gery), healing of the peri-implant tissues was better in the HA group than in the CHX group, while the effect partially reversed at midterm follow-up (6 months). They concluded that HA had a favorable action during tissue healing. Another study that compared the effectiveness of CHX + HA and CHX on the healing of implants found CHX + HA to have an additional anti-edematigenous effect after 15 days (118).

3. MATERIALS and METHODS

3.1. Study Population

The study population was consisted of 33 systemically healthy dental students of Yeditepe University Faculty of Dentistry, aged between 19-25 years, who were volunteered for this study. The study protocol was approved by the Ethical Committee of Yeditepe University School of Medicine that have their origin in the Decleration of Helsinki (Decision No: 652/2016) (Appendix I). Before the enrollment, volunteers were informed orally and recieved written information leaflet about the purpose, aim, reason, duration, possible benefits and possible adverse effects of the products used in this study (Appendix II). Subjects were then enrolled in this study according to the following inclusion criteria;

1) Male or female periodontally healthy subjects aged between 18-25
2) Presence of at least 24 natural teeth (excluding third molars)
3) No fixed or removable prostheses, and orthodontic appliances
4) No systemic disease
5) No lactation or pregnancy
6) No history of drug abuse
7) No smoking
8) No adverse reactions to CHX or HA
9) No use of systemic or topical oral antimicrobial therapy in the previous 3 months

Subjects fullfilling the inclusion criteria and willing to actively participate in this study were asked to sign a consent form prior to the study procedures (Appendix III).

3.2. Sample Size Calculation

Sample size was calculated with G* Power and Sample Size Program®[1]. Based on the data from a previous study of a 4-day non-brushing model mean difference of PI scores of 0.5921 between the test and control group was calculated. A standart deviation of 0.7 and an α error of 0.05 to obtain 80% power, 30 subjects would be sufficient for this cross-over study. In order to compensate the drop-out rate during the study period, a 10 % of drop out was considered and 33 subjects were included.

[1]www.powerandsamplesize.com/Copyright © 2013-2018 HyLown Consulting LLC • Atlanta, GA

3.3. Treatment Products

Treatment products used in this study are shown in Table 3.1.

Chlorhexidine (CHX) containing mouthwash was the positive control product in this study and the commercial brand is known as Klorhex®[2] mouthwash. It contains 0.2% CHX gluconate as an active ingredient; water, 2% glycerin as an inactive ingredient and 0.2% lemon scent and 0.02% mint scents as a flavor.

HA-containing mouthwash was the test product in this study and the commercial brand is known as Gengigel Hydrogel®[3] mouthwash. It contains 0.025% of HA and 7.5% Xylitol as an active ingredients; water, cellulose gum, alcohol, PEG40 hydrogenated castor oil, polyvinyl alcohol, polycarbophil, 2,4 dichlorobenzyl alcohol and sodium as a non-medicinal ingredients and a blend of essential oils of citromint as a flavor .

DW[4] solution was the negative control product in this study.

All of the three mouthwashes were dispensed in 200 ml identically same opaque[5] bottles and coded as A for CHX (Figure 3.1.), B for HA (Figure 3.2.) and C for DW (Figure 3.3.) to prevent any bias and the codes were not broken before the end of the study. The dispensing and the coding of the mouthwash bottles were done by a nursing sister (A.C.) in the Department of Periodontology to ensure double blindness in the study.

Table 3.1. Treatment Products Used in the Study.

Mouthwash	Active Ingredients	Code
Klorhex (CHX) positive control product	0.2% Chlorhexidine Gluconate	A
Gengigel (HA) Test product	0.025% Hyaluronic Acid 7.5% Xylitol	B
Distilled water (21) Negative control product	Distilled Water	C

[2] Klorhex® 0.2%, Drogsan, Ankara, Turkey
[3] Gengigel® 0.025%, Ricerfarma, Milano, Italy
[4] Distilled water, Aqua Medicals, Istanbul, Turkey
[5] Opaque bottles, MedDent plastics, Istanbul, Turkey

Figure 3.1. Mouthwash Bottle Coded as A for CHX.

Figure 3.2. Mouthwash Bottle Coded as B for HA.

Figure 3.3. Mouthwash Bottle Coded as C for DW

3.4. Treatment Groups

The subjects were coded with numbers as 1,...,33 and randomized equally to 3 treatment groups by computer generated-program[6].

Subjects in Treatment Group 1 (n=11) used; CHX Mouthwash A (MW/A) in the first period, HA Mouthwash B (MW/B) in the second period and DW Mouthwash (MW/C) in the third period.

Subjects in Treatment Group 2 (n=11) used; HA (MW/B) in the first period, DW (MW/C) in the second period and CHX (A) in the third period.

Subjects in Treatment Group 3 used DW (MW/C) in the first period, CHX (MW/A) in the second period and HA (MW/B) in the third period.Sequence of treatment product allocation to the traetment groups was done according to 3x3x3 Latin square cross-over design (119). The randomization and sequence of treatment product allocation of the study groups is shown in Figure 3.4.

[6]www.randomizer.org/ Copyright ©1997- 2011 by Geoffrey C. Urbaniak and Scott Plous.

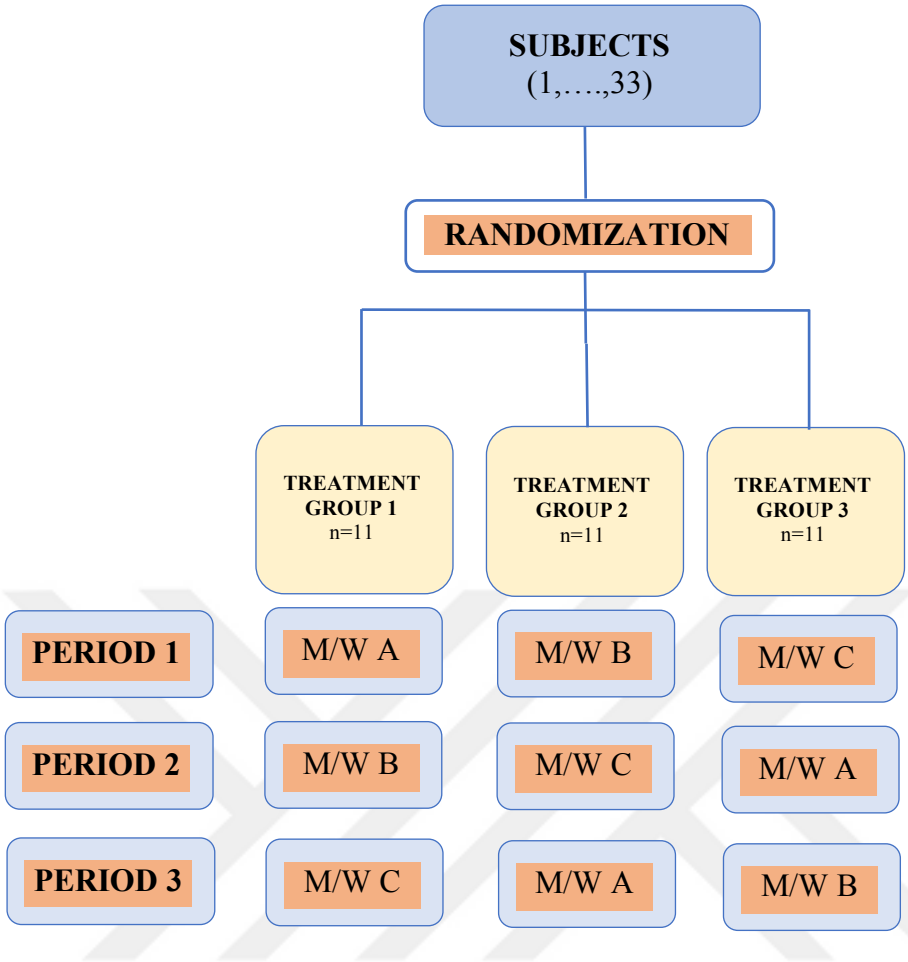

Figure 3.4. Randomization of the Subjects and Sequence of Treatment Product Allocation.

3.5. Study Design

The present study was designed as a short-term, double-blind, randomized Latin-square controlled, 4-day non-brushing, cross-over experimental study. It was consisted of pre-experimental period (7 days), first experimental period (4 days), first washout period (10 days), second experimental period (4 days), second washout period (10 days) and third experimental period (4 days). Each experimental period started on monday morning and subjects returned to clinic for the measurements on Friday morning. One of the representative chronogram of each experimental period is shown on Table 3.2. The total duration of the study was 39 days and it was performed between the dates 09.03.2017 and 18.04.2017.

Table 3.2. Chronogram of Each Experimental Week.

	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY
MORNING	GCF sampling GI PROPHYLAXIS ORAL HYGIENE CESSATION	USE 2	USE 4	USE 6	USE 8
NIGHT	USE 1	USE 3	USE 5	USE 7	GCF sampling PI GI PROPHYLAXIS COMPLIANCE QUESTIONAIRE

3.6. Interventions

On Day 1 (D1) of each experimental period, GCF samples were taken from the mesial or distal side of the first premolar teeth in each quadrant and all teeth except 3rd molars examined for GI according to Lobene Modified Gingival Index (MGI). Each subsequent full-mouth assessment lasted approximately 30 min. At the end of the full mouth assessment, all subjects received professional supra-gingival prophylaxis before they left the clinic. They were then asked to stop oral hygiene procedures for the following 4 days. During this period, the only means of oral hygiene that they were allowed was the use of the mouthwash that was allocated to them, which were prepared in identical opaque bottles. Participants were asked to rinse with 20 ml of the allocated mouthwash twice daily for 30 seconds at each time, once in the morning after breakfast, once at night before they go to sleep. Subsequent rinsing with water, drinking or eating was not allowed for 30 min after each rinsing time. Rinsing was performed at home without supervision. All instructions were given in detail verbally as well as in writing. To check for compliance, subjects were asked to register the time of use of intervention products onto a calendar record chart and asked to return bottles that contain mouthwashes.

On Day 5 (D5) of the each experimental period, GCF samples were taken from the distal and/or mesial side of the first premolar teeth in each quadrant, GI was recorded accordingto Lobene modified gingival index and PI was recorded by the Turesky Modified Quickley Hein Index system (QHI-s) after disclosing agent[7]. Finally, all

subjects received a questionnaire to evaluate their attitude towards the used product. They were questioned about their opinion of appreciation of taste, alteration of taste, comfort of use, sensitivity, numbness, duration of taste and perception of plaque control. Subjects marked a point on a 10-cm-long uncalibrated line with the negative extreme response (0) on the left and the positive extreme (10) at the right end (Visual Analogue Scale, VAS). All measurements were carried out under the same conditions and were performed by the same experienced examiner (N.A) who was blinded to the regimen. After the end of each experimental period, a wash out period of 10 days followed in which participants returned to their normal oral hygiene methods. It was assumed that they had used oral hygiene products which were given them at the beginning of the pre-experimental period by the study investigator. Only mechanical oral hygiene insturments was allowed during the washout period to eliminate the possible carry-over effects of products. The flow-chart of the study is shown in Figure 3.4.

[7]Tepe PlaqueSearch, Malmö, Sweden

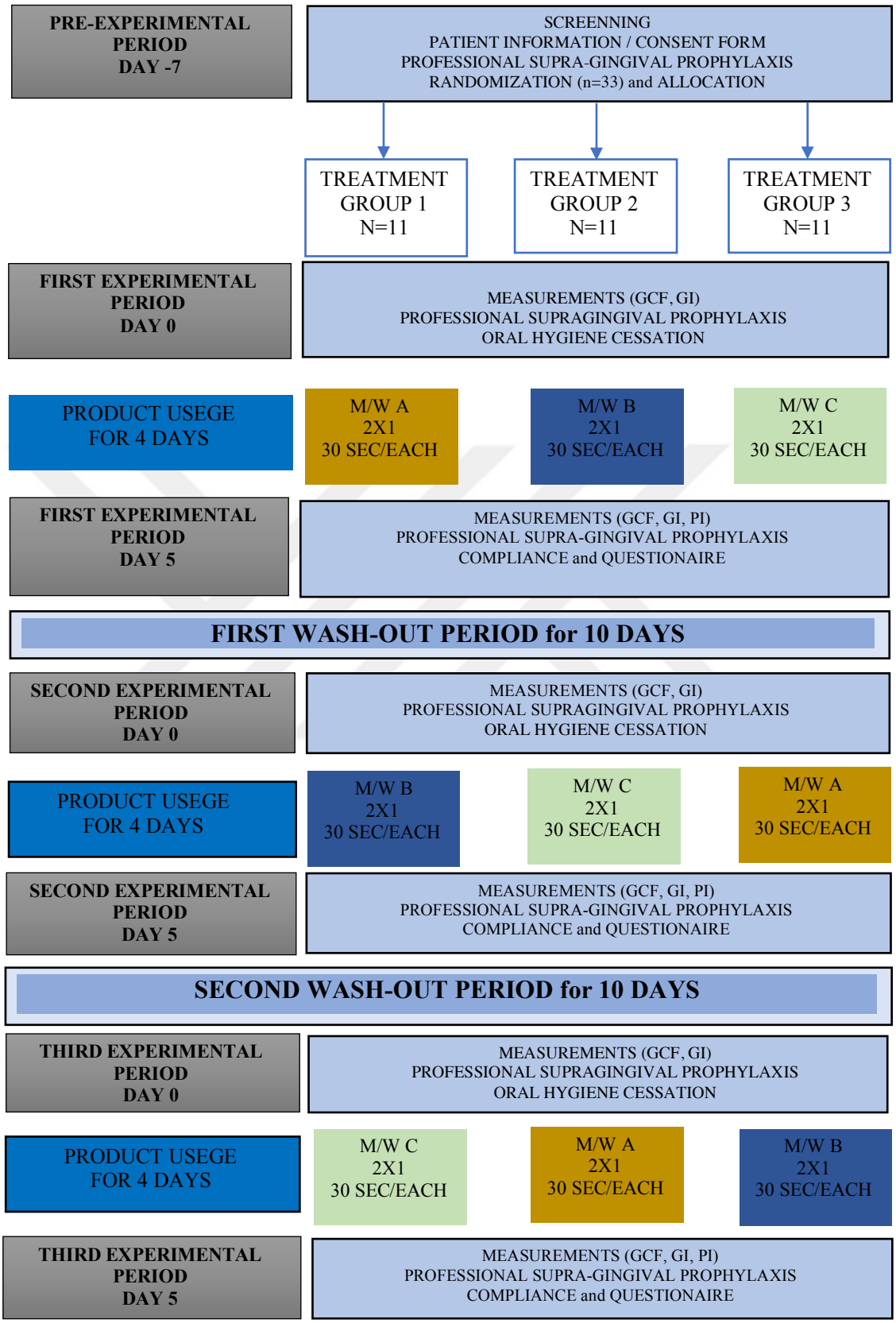

Figure 3.5. Flow-chart of the Study.

3.7. Clinical Indices and Measurements

3.7.1. Plaque Index

The mean PI was the primary outcome variable in this study. On D5 of each experimental period, a disclosing solution[7] was applied to all teeth except third molars with cotton swap and the subjects were asked to rinse with 20 ml of tap water for 15 s. Plaque was recorded at six sites per tooth (mesio-facial, mid-facial, disto-facial, mesio-lingual, mid lingual, disto-lingual) according QHI-s (120). Scoring criteria with a numerical scale were as follows;

0 = No plaque

1 = Separate flecks of plaque at the cervical margin of the tooth

2 = A thin continuous band of plaque (up to 1 mm) at the cervical margin of the tooth.

3 = A band of plaque wider than 1 mm at the cervical margin of the tooth.

4 = Plaque covering at least one-third but less than two-thirds of the crown of the tooth.

5 = Plaque covering two-thirds or more of the crown of the tooth.

The scores from the six sites of the tooth were added and divided by six to give the PI for one tooth. Adding the indices for examined teeth and dividing by the total number of examined teeth, the mean PI for the subject was obtained.

3.7.2. Gingival Index

The mean GI was assessed by The Modified Gingival Index (MGI), devised by Lobene on D1 and on D5 of the each experimental period, recorded with numbers 0-4, according to following criteria;

0= absence of inflammation;

1= mild inflammation or with slight changes in color and texture but not in all portions of gingival marginal or papillary;

2= mild inflammation, such as the preceding criteria, in all portions of gingival marginal or papillary;

3=moderate, bright surface inflammation, erythema, edema and/or hypertrophy of gingival marginal or papillary;

4= severe inflammation: erythema, edema and/or marginal gingival hypertrophy of the unit or spontaneous bleeding, papillary, congestion or ulceration.

[7]Tepe PlaqueSearch, Malmö, Sweden

The scores from the six sites of the tooth (mesio-facial, mid-facial, disto-facial, mesio-lingual, mid lingual, disto-lingual) were added and divided by six to give the mean GI score for one tooth. Adding the indices for examined teeth and dividing by the total number of examined teeth, the mean GI for the subject was obtained.

3.7.3. GCF Sampling and Volume Determination

GCF samples were collected with sterile perio-paper strips[8] (Figure 3.5.) on day 1 and day 5 of each experimental period. Samples were taken from the mesial or distal crevice of one premolar tooth from each quadrant. The sites were isolated with cotton rolls and dried with a gentle stream of air. Any visible deposits of supra-gingival plaque were removed before sampling. Periopaper strips was placed carefully into the gingival crevice until mild resistance is felt (1–2 mm into the pocket) and hold in place for 30 s (Figure 3.6.) (121). Strips contaminated by blood or exudate was excluded. Peritron 8000®[9] was used for the assessment of the GCF volume (Figure 3.7.). Periopaper® was transferred quickly to the jaws of the Periotron 8000® device to minimize evaporation errors, and the position of each peripaper was placed between the jaws in a standardized position (6 o'clock position with the black line on the Periopaper® positioned at the perimeter of the lower jaw of the device). The volume of GCF was determined by means of a previously calibrated electronic device and converted into an actual volume (μl) by reference to the standard curve.

[8]Periopaper Oraflow Inc., New York, USA
[9]Peritron 8000® Smithtown, New York, USA

Figure 3.6. Periopaper® Strips.

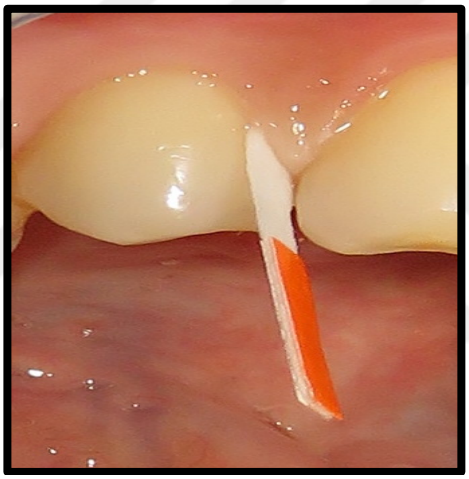

Figure 3.7. Collection of GCF Samples.

Figure 3.8. Periotron 8000® Device.

3.8. Satisfaction Questionaire

At the end of each experimental period, the satisfaction questionaire form was given to the patients to evaluate their attitudes with regard to the products used which scored by a Visual Analoque Scale (VAS) (Appendix V). The questions were evaluating the taste perception, duration of the taste, alteration of taste, sensitivity, burning sensation, dry mouth, numbness, staining and cleanliness. For each question, the subjects marked a point on a 10-cm-long line with the negative extreme response ‘0’ at the left end and the positive extreme ‘10’ at the right end. The list of the complete questions are shown in Table 3.5.

Table 3.3. Complete Questions of Satisfaction Questionaire with VAS Score (0-10).

Paraphrase	Complete Question	WITH EXTREMES (VAS)	
		FROM -0.0-	TO -10.0-
Taste perception	How was the taste of the product?	VERY BAD	VERY GOOD
Duration of taste	How long did the taste remain?	VERY SHORT	VERY LONG
Alteration of taste	How was the taste of food and drinks affected?	NEGATIVE CHANGE	POSITIVE CHANGE
Sensitivity	Did you experience sensitivity in your mouth and/or the teeth because of the product?	NOT AT ALL	VERY MUCH
Burning sensation	Did you experience a burning sensation because of the mouthwash?	NOT AT ALL	VERY MUCH
Dry mouth	Did you experience a dry mouth because of the mouthwash?	NOT AT ALL	VERY MUCH
Numbness feeling	Did you experience a numbness feeling in the mouth because of the mouthwash?	NOT AT ALL	VERY MUCH
Staining	Did you experience staining on teeth because of the mouthwash?	NOT AT ALL	VERY MUCH
Cleanliness	Did you have the feeling that your teeth were clean fort he last 4 days?	NOT AT ALL	VERY MUCH

3.9. Statistical Analysis

At the end of each experimental period, statistical analysis was performed by IBM SPSS® Statistics 22* software[10]. The compliance of parameters to the normal distribution was evaluated by Shapiro wilks test. Intra-treatment comparisons of the parameters with normal distribution evaluated with paired sample t-test whereas repeated measures analysis of variance was used for inter-treatment in-pairs and Bonferroni test was used as post hoc. Inter-treatment comparisons of the parameters without normal distribution evaluated with Friedman test and Wilcoxon signed rank test as post hoc. Statistical significance was set at $p<0.05$.

[10]IBM SPSS® Statistics 22* Software, Softonic International SA, 1997-2018, Turkey

4. RESULTS

4.1. Demographic and Baseline Datas

33 subjects (15 female, 18 male) aged between 19-25 were included in this study. The mean years of ages was 21.18±1.97. The mean PI value was 1.70±0.27 and mean GI value was 1.37±0.23 at the time of recruitment (Table 4.1).

All of the subjects completed the experimental periods and there were no missing values. Returns of each product suggested compliance with the instructions. No adverse events or adverse effects were reported and none of the subjects were excluded from the study.

Table 4.1. Demographic and Clinical Baseline Datas of the Subjects.

NUMBER of SUBJECTS	33	
GENDER	18 F (%54.5), 15 M (%45.5)	
AGE (19-25)	Mean	SD
	21.18	±1.97
PI	1.70	±0.27
GI	1.37	±0.23
PD	<3mm	

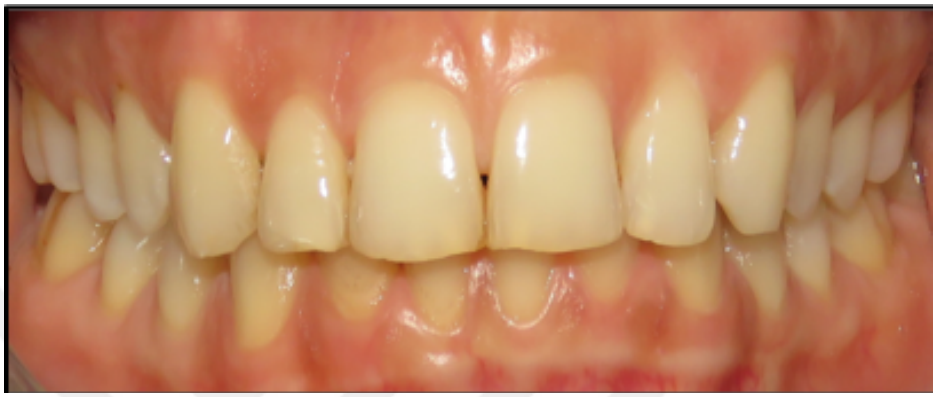

Figure 3.9. The Clinical View of CHX Treatment on D1.

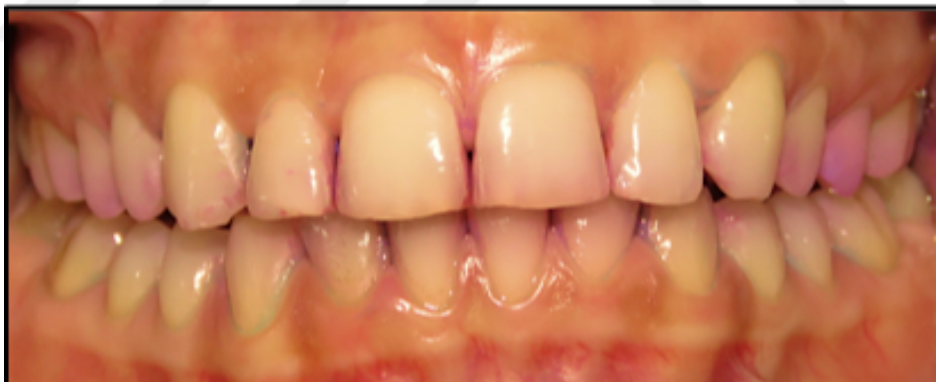

Figure 3.10. The Clinical View of CHX Treatment on D5.

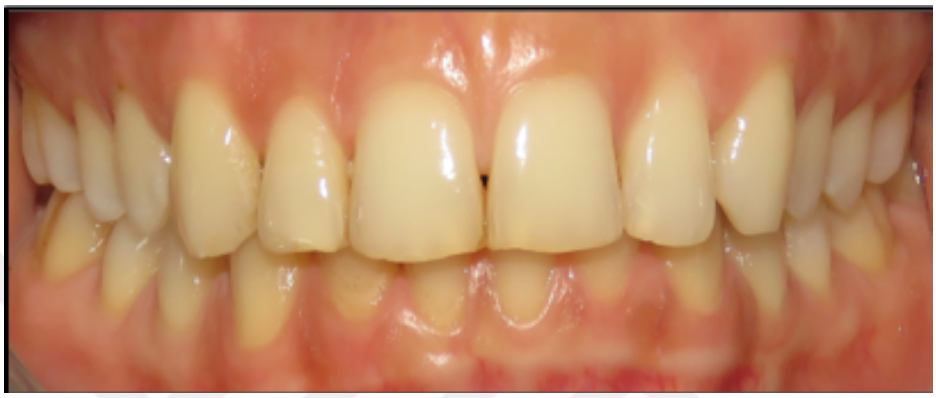

Figure 3.11. The Clinical View of HA Treatment on D1.

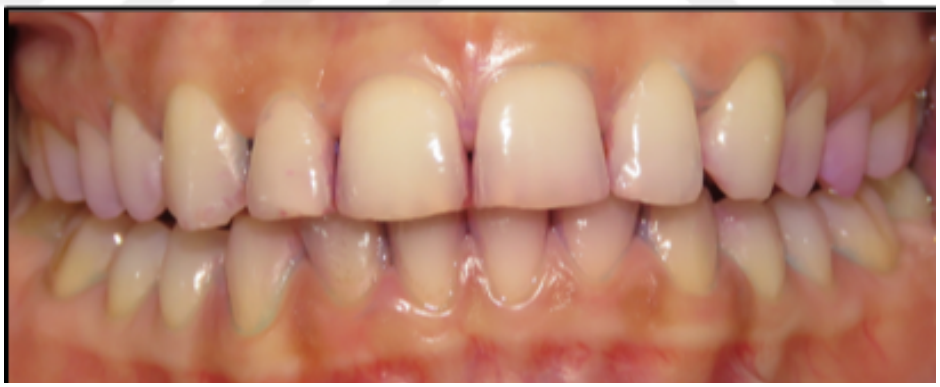

Figure 3.12. The Clinical View of HA Treatment on D5.

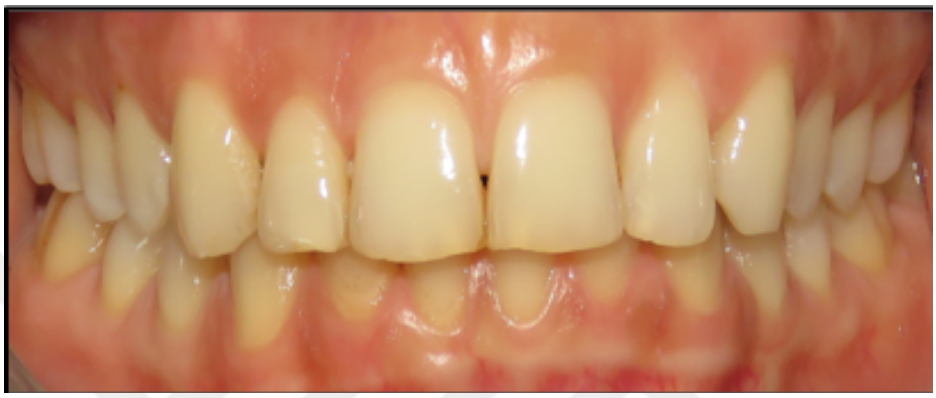

Figure 3.13. The Clinical View of DW Treatment on D1.

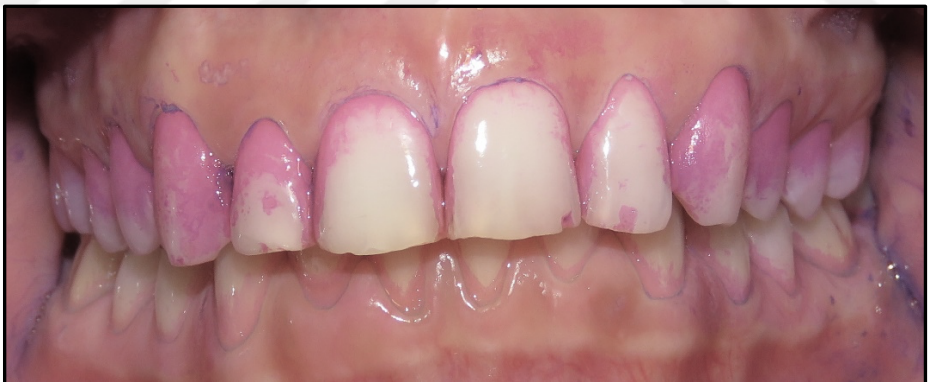

Figure 3.14. The Clinical View of DW Treatment on D5.

4.2. Clinical Results

4.2.1. Plaque Index

The mean PI value on day 5 was found 1.64±0.31 for CHX, for 1.81±0.21 for HA and 2.13±0.21 for DW treatments respectively The mean PI values and SD for CHX, HA and DW treatments on D5 are shown in Table 4.2. Inter-treatment comparisons of the mean PI values showed a statistically significant difference (p=0.000) (Table 4.2.). Subsequent comparisons of the mean PI to determine which treatment the significance is from, subsequent double comparison of the mean PI values in pairs revealed statistically significant differences between CHX and HA, CHX and DW, HA and DW treatment in favor of the CHX treatment group (p=0.048, p=0.01, p=0.01) respectively (Table 4.3.).

Table 4.2. Mean Values and Standart Deviations of PI on D5.

PI **n=33**	**CHX**	**HA**	**DW**	**p***
	(Mean±SD)	(Mean±SD)	(Mean±SD)	
D5	1.64±0.31	1.81±0.21	2.13±0.21	**0.000***

*Repeated measures analysis of variance, p<0.05

Table 4.3. Inter-treatment Comparisons of the Mean PI Values in Pairs.

PI **n=33**	**CHX vs. HA**	**CHX vs. DW**	**HA vs. DW**
	p	**p**	**p**
D5	**0.048***	**0.000***	**0.000***

*Bonferronni test, p<0.05

4.2.2. Gingival Index

On D1, mean GI values were found 0.55±0.43, 0.59±0.40, 0.51±0.30 for CHX, HA and DW treatments respectively. No significant difference was detected between the mean GI values of the treatments on D1 (p=0.729). On D5, mean GI values were detected (0.61±0.38), (0.58±0.40), (0.51±0.40) for CHX, HA and DW treatments respectively. No significant difference was detected between the mean GI values of treatments on D5 (p=0.131). All the treatments showed statistically significant increase from D1 to D5 in terms of GI values (Table 4.4.).

The mean changes of the GI values were found statistically different between the treatments. Subsequent comparisons of the changes of GI values in pairs were detected statistically significant between CHX and HA, and HA and DW treatments (Table 4.5.).

Table 4.4. Mean Values at D1, D5 and Changes in GI.

GI n=33	CHX (Mean±SD)	HA (Mean±SD)	DW (Mean±SD)	[1]p
D1	0.55±0.43	0.59±0.40	0.51±0.30	0.729
D5	0.61±0.39	0.69±0.38	0.80±0.40	0.131
CHANGE	0.06±0.11	0.11±0.11	0.29±0.25	**0.000***
[2]p	**0.005***	**0.000***	**0.000***	

[1]Repeated measures analysis of variance; [2]Paired sample t test, *p<0.05

Table 4.5. Inter-treatment Comparison of Mean GI in Pairs at D5.

GI n=33	CHX vs. HA p	CHX vs. DW p	HA vs. DW p
CHANGE	0.246	**0.000***	**0.002***

Bonferroni test , *p<0.05

4.2.3. GCF Volume

The mean value and SD of GCF volume on D1 and D5 were detected as 0.58±0.13 and 0.77±0.15 for CHX, 0.61±0.29 and 0.79±0.34 for HA, 0.65±0.27 and 0.91±0.27 for DW treatments respectively. CHX exhibited no significant difference between D1 and D5 ($p>0.05$). Both HA and DW groups showed statistically significant increases at D5 compared to D1 (Table 4.6.).

Inter-treatment comparisons of the mean GCF volume showed no statistically significant difference at D1 between the groups ($p=0.374$, $p>0.05$). Inter-treatment comparisons revealed no statistically significant differences among three groups at day 5 ($p=0.056$, $p>0.05$). The difference in the mean values of GCF between day 1 and day 5 were detected as 0.19±0.12, 0.18±0.19, 0.26±0.21 for CHX, HA and DW group respectively. Inter-treatment comparison of difference between day 1 and day 5 revealed no statistically significant difference between groups ($p=0.186$, $p>0.05$).

Table 4.6. Mean Values at D1 and D5 and Changes in GCF Volume.

GCF VOLUME	**CHX** (Mean±SD)	**HA** (Mean±SD)	**DW** (Mean±SD)	**[1]p**
D1	0.58±0.13	0.61±0.29	0.65±0.27	0.374
D5	0.77±0.15	0.79±0.34	0.91±0.27	0.056
CHANGE	0.19±0.12	0.18±0.19	0.26±0.21	0.186
[2]p	**0.000***	**0.000***	**0.000***	

[1] Repeated measures analysis of variance; [2] Paired sample t test, $p<0.05$

4.3. Satisfaction Questionaire Responses

The satisfaction questionnaire (SQ) was completed by the subjects after each experimental period and Table 4.7. shows the mean Visual Analoque Scale (VAS) scores of the subject's appreciation of the mouthwashes.

The mean values for taste perception of CHX, HA and DW treatments were 3.33±2.56, 6.01±1.95, 5.09±2.28, respectively. The taste duration of CHX, HA and DW treatments were found 6.67±1.91, 5.70±1.35, 0.42±1.25, respectively. Altered taste was scored 6.82±3.07 for CHX treatment, 4.33±1.71 for HA treatment, 0.12±0.54 for DW treatment. Sensitivity was scored 1.82±3.03 for CHX treatment, 0.79±1.34 for HA treatment and 0.12±0.41 for DW treatment. Burning sensation was 3.82±3.51, 1.15±1.58, 0±0 for CHX, HA and DW treatments respectively. Mouth dryness was scored 2.06±3.62 for CHX treatment, 0.97±1.42 for HA treatment and 0±0 for DW treatment. Numbness feeling was scored 3.18±3.18, 1.21±1.34, 0±0 in CHX, HA and DW treatments, respectively. Staining was scored as 2.36±2.61 in CHX treatment, 0.27±0.72 in HA treatment and 0.02±0.01 in DW treatment. Mouth cleanliness was scored 5.55±3.15, 4.36±1.83, 0±0 in CHX, HA and DW treatments respectively (Table 4.7.). Multiple comparisons of the evaluated parameters in SQ revealed statistically significant differences between treatments (Table 4.7.).

The comparisons of the evaluated parameters of treatments in pairs revealed significant differences in favor of HA-containing mouthwash except for taste perception parameter (Table 4.8.).

Table 4.7. The Mean VAS Scores Of the Subject's Appreciation to the Mouthwashes.

	CHX (Mean±SD)	**HA** (Mean±SD)	**DW** (Mean±SD)	[1]**p**
Taste perception	3.33±2.56	6.01±1.95	5.09±2.28	**0,002***
Taste duration	6.67±1.91	5.70±1.35	0.42±1.25	**0.000***
Altered taste	6.82±3.07	4.33±1.71	0.12±0.54	**0.000***
Sensitivity	1.82±3.03	0.79±1.34	0.12±0.41	**0.039***
Burning	3.82±3.51	1.15±1.58	0±0	**0.000***
Mouth dryness	2.06±3.62	0.97±1.42	0±0	**0.000***
Numbness	3.18±3.18	1.21±1.34	0±0	**0.000***
Staining	2.36±2.61	0.27±0.72	0.02±0.01	**0.003***
Mouth cleanliness	5.55±3.15	4.36±1.83	0±0	**0.000***
Overall first choice	6	26	1	

[1]Friedman test, *p<0.05

Table 4.8. Comparisons of the VAS Scores of the Subjects Appreciation to the Mouthwashes in Pairs.

Questionnaire Results	CHX vs. HA	CHX vs. DW	HA vs. DW
	P	p	p
Taste perception	0.016*	0.023*	0.739
Taste duration	0.032*	0.000*	0.000*
Altered taste	0.000*	0.000*	0.000*
Sensitivity	0.101	0.005*	0.009*
Burning	0.001*	0.000*	0.001*
Mouth dryness	0.001*	0.000*	0.000*
Numbness	0.000*	0.000*	0.000*
Staining	0.008*	0.010*	0.713
Mouth cleanliness	0.629*	0.000*	0.000*

Wilcoxon sign test, *$p<0.05$

5. DISCUSSION and CONCLUSION

Microbial dental plaque is a biofilm of microorganisms responsible for the onset of gingivitis and its succession to periodontitis. The primary prevention of these diseases is provided by the disruption of the colonization, proliferation and sequential layering of biofilm in order. Since gingivitis and periodontitis are a continuum of the same inflammatory disease, the daily removal of supragingival dental plaque is a major factor in the prevention of these diseases (122).

Meticulous supra-gingival mechanical biofilm control has been demonstrated to be an effective mean for preventing the initiation and/or progression of the periodontal diseases. However, for many reasons, most patients find it difficult or even impossible to comply with the exacting level of plaque removal required to obtain optimal oral health.(123). In order to control biofilms supra-gingivally and provide optimal oral health care mechanical devices and adjunctive chemical formulations are designed, developed and marketed (9, 48). Different formats are available to deliver agents for chemical plaque control: rinses, gels, dentifrices, chewing gums, aerosols, varnishes, sustained-release devices, lozenges, and irrigators (124). Among these, the antiplaque moutwashes are widely used and mostly preffered due to their number of advantages (Favorable pharmacokinetics: easier to reach the ffective dosage of the active agent; can be used independently regardless of the lack of ability of the patient to perform tooth-brushing; allows access to difficult-to-reach areas; the tonsils can be reached by gargling; easy to use and well accepted by patients) (125).

The daily usage of these chemical spragingival biofilm control agents is well supported by a scientific rationale (64, 126). Of these, chlorhexidine is certainly the most widely studied and efficient antimicrobial agent for the chemical control of the dental biofilm, and still considered as the gold standard. CHX, a bisbiguanide with prolonged substantivity and efficacy at dental and oral surfaces; approximately 12 hours . The effect of CHX on plaque microorganisms is dose dependent which shows bactericidal effect at high concentrations and bacteriostatic effect at low concentrations. CHX-containing mouthwashes formulated in different concentrations have demonstrated significant reductions in short and long-term clinical studies (94, 127) However, many reported adverse effects of CHX make scientists search for alternative chemical substances to combat the oral biofilms as effective as CHX (95). The use of hyaluronic acid within the

periodontal therapy has become a topic of great interest and is a promising agent in this field. HA has been used in periodontology as an adjunct to scaling and root planing as an antimicrobial agent when topically applied to subgingival area as a bone regenerating agent in periodontal intrabony defects for guided bone regeneration and as a graft for gingival augmentation in mucogingival surgeries (19). Recently, HA recommended as a new adjunctive plaque control agent (128). To the best of our knowledge, there is no study in the literature evaluating the effectiveness of hyaloronic acid containing mouthwash compared to CHX containing mouthwash in a cross-over, 4-day non-brushing model. From this stand point, we aimed to investigate the efficacy of HA containing mouthwash on the plaque accumulation by evaluating the clinical parameters and GCF volume in a 4-day non-brushing period.

The present study design was suggested by Addy et al. (129) and have the intention to detect the effect of antimicrobial products on new dental plaque formation in the absence of mechanical oral procedures. It consisted of the use of the tested products by the same subject during a 4-day period when all mechanical oral hygiene procedures were stopped. After this period, the subjects were examined and all measurements were recorded. In order to avoid the carry-over effects, the subject entered a washout period (10 days). Some of the studies used longer wash-out periods longer than 10 days However, Newcombe et al. reported that the 10 days washout period is convenient to eliminate the residual effects of CHX from the tissues (130). The study had a randomized double-blind design as neither the volunteer nor the researchers were aware of the composition of the products in order to avoid the bias. Randomized controlled studies provide a higher level of evidence for chemotehrapeutic agents used for chemical plaque control (126).

The plaque score was used as the main response variable. For this study, the, as QHI-s modified by Turesky et al. (131) was selected for the evaluation of the PI score. Other plaque index systems differentiate the absence or presence of the plaque that is either detectable by a dental probe or visible by the naked eye in different extent around the gingival margin (132). In these kinds of evaluations, plaque is partially destroyed by the dental probe that is run along the gingival crevice and therefore further plaque assessment can be impaired. Therefore, QHI-s was chosen for the proper assesment of the plaque accumulation in this present study (133).

CHX was used as the positive control because of its ability to reduce plaque at a strength of 0.20% due to its antibacterial properties. The results of the present study,

showed that plaque was inhibited best by CHX followed by HA-containing mouthwash and DW. Results obtained by the CHX in the present trial, are in the expected range for the activitiy of CHX with significant inhibition on plaque score (14).

HA-containing mouthwash also exhibited antiplaque activity. This antiplaque activity can be explained by the fact that HA-containing mouthwash used in the present study contains xylitol as a preservative ingredient, and xylitol itself has an antiplaque effect (134). Lack of significant data in the literature regarding the usage of HA on the effect of plaque formation makes the comparison of the results with the other studies impossible. Only one study performed by Rodrigues et al (112). In that clinical trial parallel design was used. According to the results of that trial, HA-containing mouthwash showed similar effect to CHX on the PI parameter. These controversial findings may be due to the different study designs because every individual has a different rate of plaque growth. In the present study we selected cross-over design by using the same subjects as their own controls for comparing the different treatments thus variation is reduced by elimination of inter-individual differences. This, plus the fact that the same individual is used more than once, reduces the sample size required to demonstrate a statistically significant difference between the active and the control treatments. On the other hand, the main disadvantage of this type of trials is the carry-over effect; the effect of the active agent of the test product may be carried to the following treatment product (135). So the outcomes of the study may be aaffected.

Gingival indices are based on clinical symptoms of inflammation like gingival color, contour, bleeding, extent of gingival involvement and crevicular fluid flow. Different studies have shown that bleeding on probing can cause gingival trauma and increased bleeding after provocation. Also concerns have arisen that bacteremia following invasive procedures can represent a risk for certain patients. Furthermore, bleeding sites can be obscured by blood oozing from previously probed areas to adjacent tooth surfaces that makes the assessment more difficult .There is no evidence that invasive indices are truly objective . In this study, gingival inflammation was evaluated on the marginal and papillary gingival units on scorable teeth by using MGI by Lobene et al.; which eliminated the bleeding component and increased the sensitivity at the low-end of the scoring scale. In this study, the inrease of gingival inflammation after 4 days was higher in DW treatment, than in the HA treatment, on the other hand minor changes were observed in the CHX treatment. However no significant difference was seen between the CHX and HA treatments. This is an important finding and it can be explained by the

antiinflammatory effect of HA. HA has been proven to have long-term anti-inflammatory action, showing a decrease in the amount of plaque induced ginigvitis (112). Laurent et al. stated that the anti-inflammatory action of HA is thought to be due to its scavenging action on matrix metalloproteinases and prostaglandins which are the mediators of the inflammation (131). The results of the present study with respect to the GI values are in accordance with the study which was conducted by the Rodrigues et al . They could not find any ststistical significant difference in terms of GI values between treatments. They explained that this outcome is due to the short time frame of the study (112).

Also the gingival inflammation was further assessed by the quantitative evaluation of GCF volume through using a calibrated electronic device. GCF is a serum transudate of clinically normal periodontal tissues which becomes an inflammatory exudate when the disease is clinically detectable. It was considered as an objective parameter compared to subjective clinical indices for inflammation. The collection of GCF samples before and after the usage of test and control rinses was aimed to detect the subclinical changes in the gingival tissues during the four-day study period. Some studies found a positive correlation between GCF and clinical signs of inflammation and others did not (121). Results of the present study showed no statistically significant difference in terms of GCF reduction between the treatments beacause the 4-day duration of refrained oral hygiene is not enough to assess the antigingivitis effect. In order to assess the antigingivitis effect, long-term studies such as experimental gingivitis and home-use studies are required.

As a summary, HA-containing mouthwash can target the dental plaque biofilm with different mechanisms of action by altering the biofilm formation, inhibiting the growth and vitality of microorganisms, as well as inhibiting the bacterial adhesion. In addition, it controls the nutrients by inhibiting the bacterial toxic by products controls pH by inhibiting the acid production, showing antiinflammatory, antioedamatous and antioxidant properties and induces periodontal wound healing with different mechanisms of action. This formulation can be the optimum choice for certain selected conditions where it can be an adjunctive to mechanical oral hygiene procedures. These conditions may include individuals with gingivitis, periodontitis and peri-implant mucositis. It can also be used as a soft tissue healing agent in conditions xerostomia, lichen planus, candidiasis, head and neck therapy and where CHX use is contraindicated.

Within the limits of this study, it was concluded that a mouthwash containing HA has less antiplaque effect than CHX, but it has a comparable antigingivitis effect with CHX. It has better patient acceptance, higher patient preference and does not interfere

with patient taste perception. Further randomized, controlled, long-term clinical home-use studies are necessary to evaluate the antiplaque effect of the tested formulation with microbiological and biochemical anlaysis.

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7. APPENDICES

Appendix I. Ethical Approval

Araştırmanın Açık Adı	**Hyaluronik Asit İçerikli Ağız Gargarasının Plak İnhibisyonu Üzerine Olan Etkisinin Klinik ve Mikrobiyolojik Olarak Değerlendirilmesi**
VARSA ARAŞTIRMANIN PROTOKOL KODU	

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	KOORDİNATÖR/SORUMLU ARAŞTIRMACININ UZMANLIK ALANI	Periodontoloji Anabilim Dalı Periodontoloji Anabilim Dalı			
	KOORDİNATÖR/SORUMLU ARAŞTIRMACININ BULUNDUĞU MERKEZ	Yeditepe Üniversitesi Diş Hekimliği Fakültesi Yeditepe Üniversitesi Diş Hekimliği Fakültesi			
	VARSA İDARİ SORUMLU UNVANI/ADI/SOYADI				
	DESTEKLEYİCİ				
	PROJE YÜRÜTÜCÜSÜ UNVANI/ADI/SOYADI (TÜBİTAK vb. gibi kaynaklardan destek alanlar için)				
	DESTEKLEYİCİNİN YASAL TEMSİLCİSİ				
	ARAŞTIRMANIN FAZI VE TÜRÜ	FAZ 1	☐		
		FAZ 2	☐		
		FAZ 3	☐		
		FAZ 4	☐		
		Gözlemsel ilaç çalışması	☐		
		Tıbbi cihaz klinik araştırması	☐		
		İn vitro tıbbi tanı cihazları ile yapılan performans değerlendirme çalışmaları	☐		
		İlaç dışı klinik araştırma	☒		
		Diğer ise belirtiniz			
	ARAŞTIRMAYA KATILAN MERKEZLER	TEK MERKEZ ☒	ÇOK MERKEZLİ ☐	ULUSAL ☒	ULUSLARARASI ☐

Prof. Dr. Turgay ÇELİK
Yeditepe Üniversitesi KAEK Başkanı

Araştırmanın Açık Adı	**Hyaluronik Asit İçerikli Ağız Gargarasının Plak İnhibisyonu Üzerine Olan Etkisinin Klinik ve Mikrobiyolojik Olarak Değerlendirilmesi**
VARSA ARAŞTIRMANIN PROTOKOL KODU	

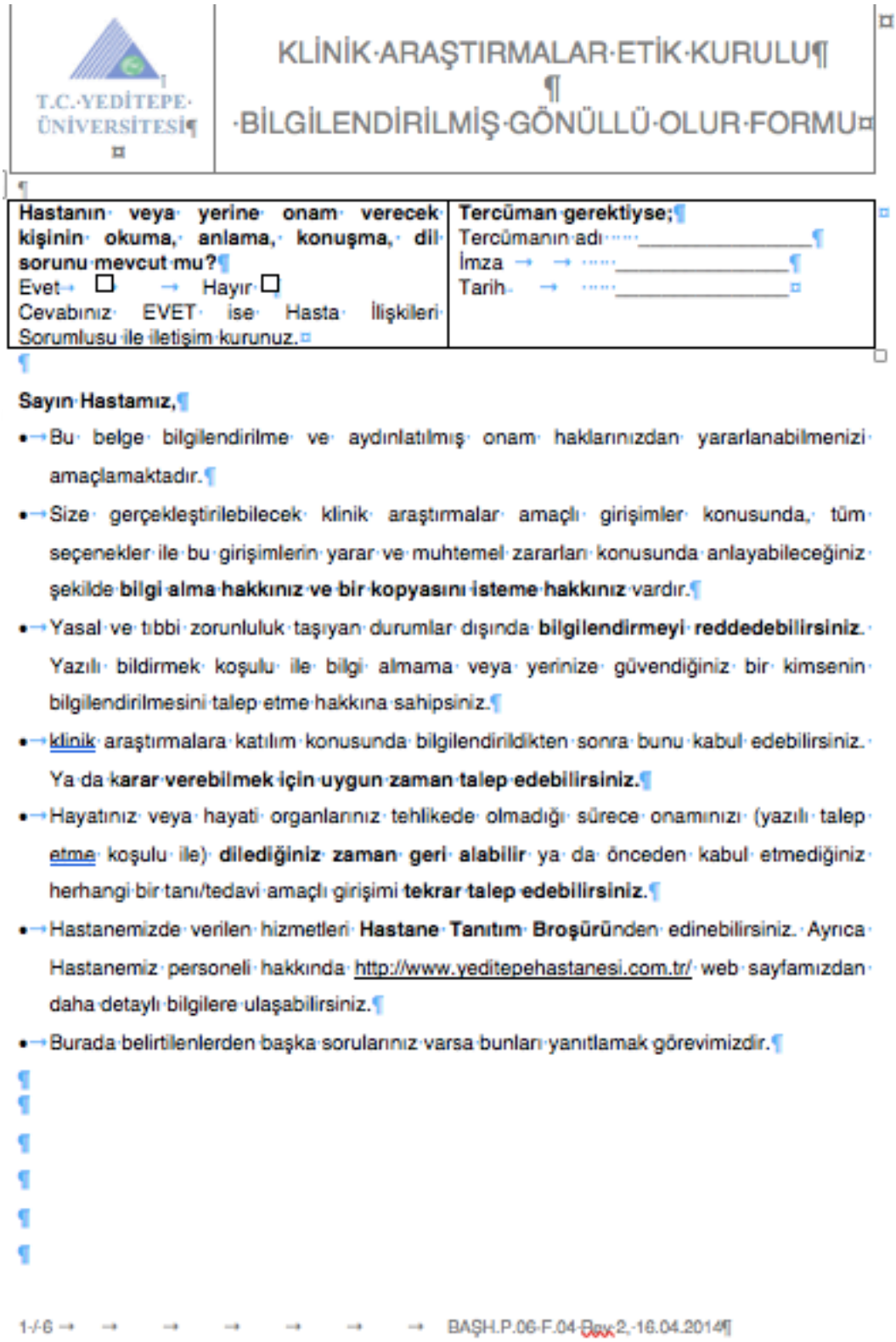
	Belge Adı	Tarihi	Versiyon Numarası	Dili
DEĞERLENDİRİLEN BELGELER	ARAŞTIRMA PROTOKOLÜ	20.07.2016		Türkçe ☒ İngilizce ☐ Diğer ☐
	BİLGİLENDİRİLMİŞ GÖNÜLLÜ OLUR FORMU	20.07.2016		Türkçe ☒ İngilizce ☐ Diğer ☐
	OLGU RAPOR FORMU	20.07.2016		Türkçe ☒ İngilizce ☐ Diğer ☐
	ARAŞTIRMA BROŞÜRÜ			Türkçe ☐ İngilizce ☐ Diğer ☐

	Belge Adı		Açıklama
DEĞERLENDİRİLEN DİĞER BELGELER	SİGORTA	☒	
	ARAŞTIRMA BÜTÇESİ	☒	
	BİYOLOJİK MATERYEL TRANSFER FORMU	☐	
	İLAN	☐	
	YILLIK BİLDİRİM	☐	
	SONUÇ RAPORU	☐	
	GÜVENLİLİK BİLDİRİMLERİ	☐	
	DİĞER:	☐	

KARAR BİLGİLERİ	Karar No: 672	Tarih: 29.06.2016
	Yukarıda bilgileri sunulan KAEK başvuru dosyası ile ilgili belgeler araştırmanın/çalışmanın gerekçe, amaç, yaklaşım ve yöntemleri dikkate alınarak incelenmiş ve Tıbbi Cihaz Klinik Araştırması olması nedeniyle; İlaç ve Biyolojik Ürünler ile Tıbbi Cihaz Klinik Araştırmaları Hakkında Yönetmelik kapsamında yer alan araştırmaların/çalışmalar için Türkiye İlaç ve Tıbbi Cihaz Kurumu'ndan izin alınması hükmü gereği; İlgili başvuru dosyasının T.C. Sağlık Bakanlığı, TITCK' a sunulmasının uygun olacağı değerlendirilmiştir.	

Prof. Dr. Turgay ÇELİK
Yeditepe Üniversitesi KAEK Başkanı

Appendix II. The Consent Form



T.C. YEDİTEPE ÜNİVERSİTESİ

KLİNİK ARAŞTIRMALAR ETİK KURULU

BİLGİLENDİRİLMİŞ GÖNÜLLÜ OLUR FORMU

Hastanın veya yerine onam verecek kişinin okuma, anlama, konuşma, dil sorunu mevcut mu? Evet ☐ → Hayır ☐ Cevabınız EVET ise Hasta İlişkileri Sorumlusu ile iletişim kurunuz.	**Tercüman gerektiyse;** Tercümanın adı ……_____ İmza → → ……_____ Tarih → → ……_____

Sayın Hastamız,

- Bu belge bilgilendirilme ve aydınlatılmış onam haklarınızdan yararlanabilmenizi amaçlamaktadır.
- Size gerçekleştirilebilecek klinik araştırmalar amaçlı girişimler konusunda, tüm seçenekler ile bu girişimlerin yarar ve muhtemel zararları konusunda anlayabileceğiniz şekilde **bilgi alma hakkınız ve bir kopyasını isteme hakkınız** vardır.
- Yasal ve tıbbi zorunluluk taşıyan durumlar dışında **bilgilendirmeyi reddedebilirsiniz.** Yazılı bildirmek koşulu ile bilgi almama veya yerinize güvendiğiniz bir kimsenin bilgilendirilmesini talep etme hakkına sahipsiniz.
- klinik araştırmalara katılım konusunda bilgilendirildikten sonra bunu kabul edebilirsiniz. Ya da **karar verebilmek için uygun zaman talep edebilirsiniz.**
- Hayatınız veya hayati organlarınız tehlikede olmadığı sürece onamınızı (yazılı talep etme koşulu ile) **dilediğiniz zaman geri alabilir** ya da önceden kabul etmediğiniz herhangi bir tanı/tedavi amaçlı girişimi **tekrar talep edebilirsiniz.**
- Hastanemizde verilen hizmetleri **Hastane Tanıtım Broşüründen** edinebilirsiniz. Ayrıca Hastanemiz personeli hakkında http://www.yeditepehastanesi.com.tr/ web sayfamızdan daha detaylı bilgilere ulaşabilirsiniz.
- Burada belirtilenlerden başka sorularınız varsa bunları yanıtlamak görevimizdir.

1-/-6 → → → → → → → BAŞH.P.06-F.04-Rev.2,-16.04.2014

TANIMLAMA

Araştırmanın Adı / Protokol numarası

Farklı içerikli ağız gargaralarının plak oluşumunu önleyici etkinliklerinin klinik olarak 4 günlük plak akümülasyon modeli ile değerlendirilmesi.

Araştırma Konusu

Farklı içerikli ağız gargaralarının plak oluşumunu önleyici etkinliklerinin değerlendirilmesi

Araştırmaya Katılımcı Sayısı

33

Bu araştırmanın

Amacı

→ Bu çalışmada, farklı içerikli ağız gargaralarının, gönüllülerin diş fırçalamayı gerçekleştirmediği durumlarda, plak oluşumunu önleyici etkinliklerinin 4 günlük plak akümülasyon modeli ile klinik olarak değerlendirilmesi amaçlanmaktadır. Araştırmada ayrıca bireylerin farklı içerikli gargaraları kullanımları sonrasında bu gargaralar ile ilgili deneyimledikleri yan etkiler değerlendirilecektir.

Süresi

→ 10 hafta

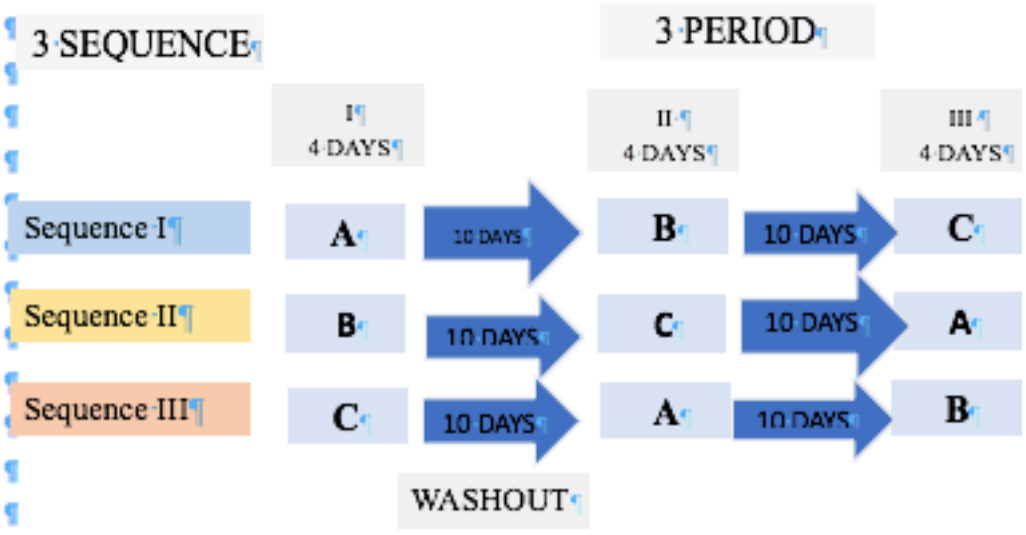
İzlenecek Yöntem / Yöntemler

Bu çalışma randomize kontrollü, çift kör, cross-over, 4 gün plak akümülasyon modeli, klinik çalışmadır. Çalışmamıza Yeditepe Üniversitesi Diş Hekimliği Fakültesi öğrencilerinden gönüllü olan, sistemik ve periodontal açıdan sağlıklı bireyler dahil edilmesi planlanmaktadır. Onamları alınan ve çalışmaya dahil edilme kriterlerine uygun gönüllülerin herbiri 1, den 33 e kadar numara ile kodlanacaktır. Gönüllüler ve araştırmadan sorumlu hekim kullnılan gargara şişelerinde hangi formulasyonun olduğunu bilmeyecektir ve önceden hazırlanmış kutularda kendilerine verilecekti, kodlanan hastalar 3 ayrı sıralamaya ait 3 ayrı gruba randomize edilecektir. Gönüllülerin herbirine çalışmaya başlanması planlanan tarihten 1 hafta önce supragingival profilaksi ve polisaj işlemleri yapılacak, oral hijyen eğitimi verilecek ve çalışma periodları arasındaki arınma dönemlerinde kullanmaları için herbiri aynı olan diş fırçası ve diş macunu dağıtılacaktır. Herbir çalışma periodunun 1 Gününde supragingival profilaksi ve polisaj işlemini takiben, gingival index ölçümü yapılacak, dişeti oluğu sıvısı herbir kadrandaki 1 dişin dişeti cebi içerisine 30 sn süreyle yerleştirilen periopaper yardımıyla alınacak ve periotron 8000 cihazı yardımıyla hacimsel değeri ölçülecektir. Ölçümlerin skorlanması sonrasında hastalara daha önceden atanmış olan gargaraların nasıl

kullanılacağı hakkında bilgi verilecek ve 4 gün boyunca kendilerine atanan gargaralardan başka hiçbir oral temizlik yapmamaları söylenecektir. Gargaraların kullanımı sabah 20ml/30 sn. kahvaltıdan sonra ve akşam yatmadan hemen önce 20ml/30 sn. şeklinde olacaktır. Gargaraların kullanımından sonra hastalar 30 dak. Boyunca hiçbir yiyecek ve içecek tüketmeyecektir. 4. Günlük gargara kullanımı sonunda katılımcılardan şişeleri beraberinde getirmeleri istenecektir. Katılımcılardan dişeti oluğu sıvısı örnekleri alınarak Plak ve gingival index skorlamaları yapılacaktır. Hastalar kliniği terketemeden önce supragingival profilaksi uygulanacak ve kullandıkları garagaralar hakkında sorular içeren bir anket doldurmaları istencektir. Bu 4 günlük çalışma periodondan sonra katılımcılar 10 günlük arınma dönemlerinde kendi ağız hijyen alışkanlıklarına geri döncektir.

Bu çalışma bir DOKTORA TEZİ araştırmasıdır.

1 den 33 e kadar kodlanan katılımcıların 3 farklı gruplara randomizasyonu bilgisayar programı ile yapılmıştır. Randomizasyon şeması aşağıdaki gibidir.



Herbir grup n=11, çalışmanın her bir periodunun 1. Gününde farklı gargara ile çalışmaya başlayacaktır. Buna göre;

1. Periodda ; Grup 1 (n=11) A içerikli gargarayı, grup 2 (n=11) B içerikli gargarayı, grup 3 (n=11)C içerikli gargarayı kullanacaktır.
2. Periodda Grup 1 (n=11) B içerikli gargarayı, Grup 2 (n=11) C içerikli gargarayı, Grup 3 (n=11)A içerikli gargarayı kullanacaktır.
3. Periodda grup 1 (n=11) C ; grup 2 (n=11) A içerikli , grup 3 (n=11) C içerikli gargarayı kullanacaktır.

Herbir çalışma periodu 4 günlük gargara kullanımını ifade etmektedir. Çalışma periodları arasında 10 günlük arınma periodları vardır ve bu dönemde katılımcılar kendi ğız hijyenlerini uygulayacaktır.

Araştırma Sonunda Beklenen Fayda

Hastaların diş fırçalamayı gerçekleştiremediği durumlarda, periodontal hastalık oluşumunda primer etyolojik faktör olan dental plağın diş ve çevre dokularda oluşumunun önlenmesi beklenmektedir.

Alternatif Tedavi Veya Girişimler

Araştırma Sırasında Karşılaşılabilecek;

Riskleri	*Rahatsızlıklar*
a)	a)
b)	b)
c)	c)
d)	d)
e)	e)
f)	f)
g)	g)

T.C. YEDİTEPE ÜNİVERSİTESİ	KLİNİK ARAŞTIRMALAR ETİK KURULU BİLGİLENDİRİLMİŞ GÖNÜLLÜ OLUR FORMU

Risk / rahatsızlık durumlarında yapılması gerekenler

Aşağıdaki özel durumlara ait katılımcı var mı?

	EVET*	HAYIR
Çocuk		++
Mahkum		++
Gebe		++
Mental yetersizlik		++
Sosyoekonomik eğitim olarak yetersiz		++

*Ancak çocuklarda, hamilelik, lohusalık ve emzirme dönemlerinde ve kısıtlılık durumunda; gönüllüler yönünden araştırmadan doğrudan fayda sağlanacağı umuluyor ve araştırma gönüllü sağlığı açısından öngörülebilir ciddi bir risk taşımıyor ise, usulüne uygun bir şekilde alınmış bilgilendirilmiş gönüllü olur formu ile birlikte ilgili etik kurulun onayı ve Bakanlık izni alınmak suretiyle araştırmaya izin verilebilir.

T.C. YEDİTEPE ÜNİVERSİTESİ	KLİNİK ARAŞTIRMALAR ETİK KURULU BİLGİLENDİRİLMİŞ GÖNÜLLÜ OLUR FORMU

ONAM (RIZA)

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. Bu durumda hastanenin çalışma düzeni ve hastalara verilen bakımda aksaklık olmayacağı konusunda bilgilendirildim. Bu araştırmaya katılırken zorlama, maddi çıkar ve ast üst ilişkisine dayalı herhangi bir baskı olmaksızın bu çalışmaya katıldığımı beyan ederim. Bu bilimsel çalışmanın devamı esnasındaki süreçle ilgili olarak ayrıca eklenen çalışma protokolü ile bilgilendirildim.

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

Gerekiyorsa Olur İşlemine Tanık Olan Kişinin Adı / Soyadı / İmzası / Tarih

Gerekiyorsa Yasal Temsilcinin Adı / Soyadı / İmzası / Tarih

24 Saat ulaşılabilir iletişim bilgiler

Araştırma Sorumlusu: Begüm Atalay
05322083502

Appendix III. The Written Instruction Form

T.C. YEDİTEPE ÜNİVERSİTESİ	KLİNİK ARAŞTIRMALAR ETİK KURULU BİLGİLENDİRME FORMU

Değerli katılıcımcı, araştırma boyunca aşağıda size önerilen talimatları dikkatlice okuyup, uygulamanızı öneriyoruz. Buna göre,

1. Araştıma süresi boyunca, araştırmaya dahil edildiğiniz tarihte sizlere verilen diş fırçası, diş macunu ve dişipi haricinde başka hiçbir oral hijyen ajan kullanmayınız ve dişlerinizi her gün 2 defa 2 dakika boyunca fırçalayınız.
2. Çalışma periodları dahilinde (4 günlük) sadece sizlere dağıtılan gargaraları kullanınız ve bunun haricinde başka hiç bir şekilde oral hijyen uygulaması yapmayınız. Sizlere dağıtılan gargaraların kullanımı, sabah kahvaltıdan sonra 20ml gargara ile 30 saniye boyunca ve akşam yatmadan önce 20ml gargara ile 30 saniye boyunca ağzını çalkalayınız ve çalkalamadan sonra 30 dakika boyunca hiç bir yiyecek ve içeçek tüketmeyiniz.
3. Arınma periodları dahilinde (10 günlük) sizlere verilen dişmacunu, diş fırçası ve dişipi haricinde herhangi kimyasal içerikli gargara,sprey, jel uygulaması yapmayınız.

Çalışma süresi boyunca herhangi bir sistemik antibiyotik kullanımını mutlaka bildiriniz.

Appendix IV. Clinical Assessment Form

Patient code: Mouthwash: Period:

1. PLAQUE INDEX

… UPPER JAW

BUCCAL	**MWC D5**															
	MWB D5															
	MWA D5															
		17	**16**	**15**	**14**	**13**	**12**	**11**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**TOTAL**
PALATAL	**MWA D5**															
	MWB D5															
	MWC D5															

.. .. LOWER JAW

FACIAL	**MW/C D5**															
	MW/B D5															
	MW/A D5															
		17	**16**	**15**	**14**	**13**	**12**	**11**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**TOTAL**
LINGUAL	**MW/A D5**															
	MW/B D5															
	MW/C D5															

2. GINGIVAL INDEX

UPPER JAW

BUCCAL	MW/C D-5															
	MW/C D-0															
	MW/B D- 5															
	MW/B D-0															
	MW/A D-5															
	MW/A D-0															
		17	16	15	14	13	12	11	21	22	23	24	25	26	27	TOTAL
PALATAL	MW/A D-0															
	MW/A D-5															
	MW/B D-0															
	MW/B D-5															
	MW/C D-0															
	MW/C D-5															

LOWER JAW

FACIAL	MW/C D5															
	MW/C D-0															
	MW/B D-5															
	MW/B D-0															
	MW/A D-5															
	MW/A D-0															
		17	16	15	14	13	12	11	21	22	23	24	25	26	27	TOTAL
LINGUAL	MW/A D-0															
	MW/A D-5															
	MW/B D-0															
	MW/B D-5															
	MW/C D-0															
	MW/C D-5															

3. GCF VOLUME

	Tooth no and side	GCF vol.	Tooth no and side	GCF vol.	Tooth no and side	GCF vol.	Tooth no and side	GCF vol.	TOTAL
MW/ A D 0									
MW/ A D 4									
MW/ B D 0									
MW/ B D 4									
MW/ C D 0									
MW/ C D 4									

Appendix V. Satisfaction Questionaire Form Evaluated With VAS

Patient code: Mouthwash: Period:

How was the taste of the product?

Very bad										Very good
0	1	2	3	4	5	6	7	8	9	10

How long did the taste remain?

Very short										Very long
0	1	2	3	4	5	6	7	8	9	10

How was the taste of food and drinks affected?

Not at all										Very much
0	1	2	3	4	5	6	7	8	9	10

Did you experience sensitivity in your mouth and/or the teeth because of the product?

Not at all										Very much
0	1	2	3	4	5	6	7	8	9	10

Did you experience a burning sensation because of the mouthwash?

Not at all										Very much
0	1	2	3	4	5	6	7	8	9	10

Did you experience a numbness feeling in the mouth because of the mouthwash?

Not at all										Very much
0	1	2	3	4	5	6	7	8	9	10

Did you experience staining on teeth because of the mouthwash?

Not at all										Very much
0	1	2	3	4	5	6	7	8	9	10

Did you have the feeling that your teeth were clean for the last 4 days?

Not at all										Very much
0	1	2	3	4	5	6	7	8	9	10

8. CIRRICULUM VITAE

Personal Information

Name	BEGUM	**Surname**	ATALAY
Place of Birth	SAMSUN	**Date of Birth**	31.10.1984
Nationality	TURKEY	**TC Identification Number**	23984523006
E-mail	bgmatly@gmail.com	**Tel**	05322083502

Education Degree

Degree	Area	Name of the Graduated Organization	Graduation Year
Doctorate	Periodontology	**Yeditepe University**	
Bachelors	-	**Yeditepe University**	2009
High School	-	**Samsun Anatolian High School**	2002

Languages	**Exam Notes**
Turkish	Mother Language
English	IELTS Total score 7.2

TAST	Kurum	**(YEAR-YEAR)**
General Practitioner	Kartal Kızılay Hospital	2009-2010
General Practitioner	In private practice	2011-2013

Computer Information Programme

Program	**Using Skills**

Certificates
