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YEDITEPE UNIVERSITY

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DEPARTMENT OF PERIODONTOLOGY

CLINICAL EVALUATION OF THE PLAQUE INHIBITORY EFFECT OF ALOE VERA-CONTAINING MOUTH RINSE IN A 4-DAY PLAQUE REGROWTH MODEL

DOCTOR OF PHILOSOPHY THESIS

DENTIST

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APPROVAL

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This study have approved as a Doctorate Thesis in regard to content and quality by the Jury.

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APPROVAL

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 13..04...2018.....and numbered.2018/07....04

Prof. Dr. Bayram YILMAZ

Director of Institute of Health Sciences

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.

Date

23.03.2018

Signature

Name Surname

NESRIN EL-NAIHOUM

DEDICATION

With my deepest gratitude, warmest affection and greatest hamble

This thesis is dedicated to

My beloved parents, ***Mr. Mohammed El-Naihoum*** *and* ***Mrs. Najat Qunibber***

My lovely son ***Ferro***

and

My dearest Siblings

Souffian, Dina , Esra, Wanis and Hana

for everything that gave it to me

I could never have done this work without their full faith, limitless support, constant encouragement, and infinite love

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS AND ABBREVIATIONS

ADA	American Dental Association
ALV	Aloe vera
ALV+ClO_2	Aloe vera-Chlorine Dioxide Combination Mouth Rinse
CAL	Clinical Attachment Level
CFU	Colony Forming Unit
CHX	Chlorhexidine
ClO_2	Chlorine Dioxide
CMO	Citrus and Mint Oils
CoQ10	Coenzyme Q10
CP	Chronic Periodontitis
CPC	Cetylpyridinium Chloride
D0	Baseline or Day Zero
D4	The Fourth Day or Day Four
DNA	Deoxyribonucleic Acid
DW	Distilled Water
EO	Essential oils
EPA	Environmental Protection Agency
FDA	Food and Drug Administration
GCF	Gingival Crevicular Fluid
GI	Gingival Index
GIT	Gastrointestinal Tract
Gram -	Gram Negative
Gram +	Gram Positive
H_2O_2	Hydrogen Peroxide
IL	Interleukin
MGI	Modified Gingival Index
ml	Milliliter
µL	Microliter
mm	Milimeter
$O^{\cdot}$	Molecular Oxygen
OHI	Oral Hygiene Instruction
PD	Probing Depth

PI	Plaque Index
PMN	Polymorphonuclear Leukocytes
ppm	Parts per Million
QHI	Quigley-Hein Plaque Index
REB	Research Ethics Boards
RNA	Ribonucleic Acid
SD	Standard Deviation
SEM	Scanning Electron Microscope
SQ	Satisfaction Questionnaire
TNF	Tumour Necrosis Factor
tx	Treatment Period
tx_1	The First Treatment Period
tx_2	The Second Treatment Period
tx_3	The Third Treatment Period
VAS	Visual Analogue Scale
VSC	Volatile Sulfur Compounds
XYL	Xylitol
yrs.	Years
Zn^{+2}	Zinc ion
ZOI	Zone of Inhibition

ABSTRACT

EL-NAIHOUM, N. (2018). Clinical Evaluation of the Plaque Inhibitory Effect of Aloe Vera-Containing Mouth Rinse in a 4-Day Plaque Regrowth Model. Yeditepe University, Institute of Health Sciences, Department of Periodontology, Periodontology Doctorate Programme, Ph.D. Thesis, Istanbul.

The unveiling of an alternative chemotherapeutic agent to chlorhexidine formulations with the same efficacy and less adverse effects is necessitated for chemical plaque control and treatment of gingivitis. The aim of this study was to evaluate the clinical efficacy and patient perception of a combination mouth rinse containing aloe vera and chlorine dioxide (ALV+ClO_2) as active ingredients compared to 0.2% chlorhexidine gluconate (CHX) and distilled water (DW) in a 4-day plaque regrowth model with a cross-over design. Total of 33 systemically and periodontally healthy subjects were included and randomly assigned into one of the three treatments that have different sequences with a 10-day washout period. Subjects were asked to refrain from mechanical oral hygiene and only rinse with the allocated mouth rinse twice a day during the four-day period. Plaque index (PI), gingival index (GI), and gingival crevicular fluid (GCF) volume were evaluated at baseline and the fourth day of each period. Satisfaction questionnaire was performed at the end of each treatment period. Regarding the primary outcome, statistically significant changes in PI scores were detected between the treatments (ALV+ClO_2), CHX and DW ($p<0.05$) in favor of the CHX rinse. Although VAS score of (ALV+ClO_2) was less than CHX in terms of mouth cleanliness, it had better taste perception and subject preference with less negative impact on mouth dryness, sensitivity, burning, numbness and no tooth staining compared to CHX. It was concluded that (ALV+ClO_2) rinse has less plaque-inhibitory effect than that of CHX, but has a better patient perception and preference.

Keywords: chemotherapeutic agents, aloe vera, chlorine dioxide, chlorhexidine, plaque regrowth model.

ABSTRACT (Turkish)

EL-NAIHOUM, N. (2018). Aloe vera İçeren Ağız Gargarasının, Plak Inhibisyonu uzerine Olan Etkilerinn 4 Günülük Plak Akümülasyon Modelinde Klinik Olarak Değerlendirilmesi. Yeditepe Üniversitesi Sağlık Bilimleri Enstitüsü, Periodontoloji Anabilim Dalı, Doktora Tezi, İstanbul.

Plağa bağlı diş eti iltihabının önlenmesi ve tedavisi için kimyasal plak kontrolünde altın standart olarak kullanılan klorheksidine alternatif bir kemoterapötik ajanın geliştirilmesi, yan etkileri önleme bakımından gereklidir. Bu çalışmada, aktif madde olarak başlıca aloe vera ve klorin dioksit içeren gargara formülasyonunun (ALV+ClO_2) klinik etkinliğinin ve hasta memnuniyetinin, 0.2%'lik klorheksidin glukonat (CHX) ve distile su (DW) gargaraları ile 4-günlük plak akümülasyon modelinde karşılaştırılması amaçlandı. Çalışmaya dahil edilen sistemik ve periodontal açıdan sağlıklı 33 birey rastgele olarak farklı gargara kullanım sırasına sahip 3 tedavi periyoduna ayrıldı. Bu bireylerden 4 gün boyunca mekanik plak kontrolü işlemlerinden uzak durmaları ve yalnızca kendilerine verilen gargaraları kullanarak günde iki kez ağızlarını çalkalamaları istendi. Her tedavi periyodunun başlangıcı ve 4. gün sonunda plak indeksi (PI), gingival indeks (GI) ve diş eti oluğu sıvısı (DOS) hacmi ölçüldü ve 4.günün sonunda memnuniyet anketi uygulandı. Tedavi periyodları arasındaki 10 günlük arınma döneminde bireylerden günlük mekanik plak kontrolü alışkanlıklarına tekrar dönmeleri istendi. Çalışmanın sonunda PI'da, ALV+ClO_2, CHX ve DW tedavilerinde çoklu kıyaslama ve ikili karşılaştırmalarda CHX grubu lehine istatistiksel olarak anlamlı farklılıklar tespit edildi ($p<0.05$). Hasta algısına göre temizlik hissi açısından CHX, ALV+ClO_2 ye göre daha yüksek puanlanırken, ağız kuruluğu, dişlerde renkleşme, hassasiyet, yanma, uyuşukluk hissi ve tad duyusunda değişiklik parametrelerinde ALV+ClO_2, CHX'e göre anlamlı derecede daha yüksek puanlandı ($p<0.05$). Sonuç olarak, ALV+ClO_2 gargarasının plak inhibisyonu açısından CHX ile karşılaştırıldığında daha az etkili olduğu tespit edildi. Bununla birlikte, ALV+ClO_2, CHX'e göre daha çok kabul görüp tercih edildi.

Anahtar kelimeler: kemoterapötik ajan, aloe vera, klorin dioksit, kloroheksidin, plak akümülasyon modeli.

1. INTRODUCTION AND AIM OF THE STUDY

Periodontal diseases along with dental caries are considered as the most prevalent diseases of the oral cavity. These diseases are associated with oral microorganisms that organized as a complex microbial community in a form of dental biofilm (1, 2). Dental biofilm, also known as dental plaque, starts to form on teeth surfaces immediately after tooth cleaning either by a dental professional or individual home care (3). The developing biofilm releases a variety of biologically active products. These products diffuse into the surrounding gingival tissues and initiate an inflammatory host response that results in the clinical manifestation of gingivitis (4). When the biofilm left to accumulate without the establishment of oral hygiene methods or any other intervention, gingivitis is established after 2-3 weeks of plaque accumulation (2, 5). If gingivitis left untreated, it may progress to periodontitis that characterized by the formation of periodontal pockets, attachment and bone loss, and leading eventually tooth loss (6).

Plaque biofilm control is the key to the prevention and treatment of gingivitis, periodontitis and dental caries (7). It is causally directed toward their primary etiologic factor: the pathogenic microflora that colonizes the tooth surfaces and forms dental plaque biofilm (8). However, controlling periodontal diseases by eliminating pathogenic flora is, as yet, impossible (9). Since it is difficult to achieve the complete plaque removal, the prevention of disease can be achieved by reducing the quantity of dental plaque below the threshold level of disease or by changing the quality of the plaque toward more protective composition (10). Plaque biofilm control is done either mechanically or chemically by self-care or by professionals (8). Some factors can limit the success of mechanical plaque control; such as the presence of inaccessible areas, inadequate skills, poor motivation and lack of compliance (9). Therefore, chemotherapeutic agents in a form of mouth rinses, gels, and dentifrices are introduced as an adjunct to mechanical means to overcome these limitations (11). Many antimicrobial agents have been evaluated with respect to the supragingival plaque control. They can be categorized generally into bisbiguanides, quaternary ammonium compounds, phenolic agents, other antiseptics, oxygenating agents, metal ions, natural products and miscellaneous agents (12). Chlorhexidine gluconate (CHX) is a bisbiguanide which is considered as the gold standard for chemical plaque control for being the most effective antiplaque agent that demonstrated its clinical efficacy in short and long-term studies (13). CHX provides a

long-lasting bacteriostatic and bactericidal effects. It inhibits the bacterial colonization of plaque. Despite the efficacy of CHX, it has many adverse effects reported in works of literature (14). To overcome these adverse effects the researchers are still seeking an alternative to CHX that would exert the same efficacy of CHX without its adverse effects (15,16).

The use of natural and herbal products has increased recently as a fast emerging trend since they provide safe, effective and economical alternative therapy in medicine and dentistry (17). They have been recently investigated more thoroughly as promising agents for the prevention of oral diseases, particularly plaque-associated diseases (18). Among these herbal agents, Aloe vera (ALV) which is a medicinal plant that also known as *Aloe barbadensis*. Numerous studies on ALV demonstrated the antiviral, antibacterial, antifungal, analgesic, antiinflammatory, antioxidant and wound healing properties which could be attributed to its multiple biologically active components (19). It inhibits the growth of diverse oral microorganisms, such as *Streptococcus mutans*, *Streptococcus sanguis*, *Actinomyces viscosus*, and *Candida albicans* (20). Although ALV gels demonstrated an inhibitory effect on plaque and gingivitis (21-28), a limited research available to support the recommendation of ALV gel over other antiseptic mouth rinses to control plaque and gingival diseases (29, 30).

Chlorine dioxide (ClO_2) is an antiseptic agent that frequently tested for its efficacy in the reduction of oral malodor (31). It is a molecular free radical found in chlorite solutions (32). It acts as a strong oxidizing agent, which consumes oral substrates containing cysteine and methionine, preventing the production of volatile sulfur compound (VSC); the cause of oral malodor (31). Its effectiveness in suspension of periodontal pathogens had been reported (33-35). A limited number of studies on the antiplaque and antigingivitis effects of ClO_2 have exhibited promising results (15, 16, 36-39). The incorporation of ClO_2 in its anionic form into several current oral health care products; represents an effective advance in the prevention and therapeutic measures of periodontal diseases, and in the maintenance of a high level of oral hygiene (40).

Zinc (Zn^{+2}) is added in mouth rinse and toothpaste formulations as an antibacterial agent since the studies revealed its inhibitory effect on the development of dental plaque, calculus, oral malodor and gingivitis. Its antimicrobial activity against different bacteria such as *S. mutans* has been reported and it was due to its ability to inhibit the process of

glycolysis (41, 42). Additionally, it has exhibited a synergistic effect when it is combined with other antiseptics (43).

Xylitol (XYL) is a natural and non-cariogenic sweetener. It acts as a cariostatic and an antiplaque agent by affecting the biofilm formation via decreasing *S. mutans* counts, and the amount and adhesivity of plaque. Although XYL has exerted a synergistic effect when it added to other oral product like probiotics, cetylpyridinium chloride (CPC), and CHX, limited supporting studies are available (44, 45).

Essential oils such as citrus oils, mint oil (CMO) are added to oral care products as a flavoring agent, but they also demonstrated antibacterial and antioxidant activities (46). Their bactericidal effect on *S. mutans* has been reported (47, 48).

Mouth rinses may contain one or more therapeutic agents as the active ingredient (49). The synergistic combination of two or more agents can overcome toxicity and other adverse effects associated with high doses of a single agent. Also, these combinations allow using low doses of the therapeutic agents and provide a multi-target mechanism (50).

Even though the antimicrobial action of ALV, ClO_2, Zn^{+2}, XYL, and CMO has been tested *in vitro* (20, 33-35, 47, 48, 51-61) and *in vivo* (29, 30, 32, 40, 41), a considerable number of studies conducted to investigate the plaque inhibitory effect of each agent separately (15, 16, 21-28, 36-39, 43, 45). However, no plaque regrowth study has been conducted on a mouth rinse containing a combination of these agents together. Studies regarding their combined effect on dental plaque biofilm are required.

The aim of the present study was to clinically evaluate a combination mouth rinse containing aloe vera, chlorine dioxide, zinc, xylitol, and citrus and mint oils in comparison to 0.2% CHX gluconate and distilled water (DW) rinses in terms of plaque inhibition, gingival inflammation, volumetric changes in gingival crevicular fluid (GCF), satisfaction questionnaire (SQ), compliance, oral and soft tissue status, and adverse effects in a 4-day plaque regrowth model, using a double blind, randomized, controlled clinical trial.

2. LITERATURE REVIEW

Periodontal diseases are the pathological manifestations of the host response against the bacterial challenge of the dental biofilm at the tooth/gingival interface. They are characterized by the inflammation and destruction of the tooth-supporting structures that eventually leads to the loss of affected teeth (62, 63). Plaque-induced gingivitis and chronic periodontitis are the most commonly occurring forms of periodontal diseases (64, 65). Plaque-induced gingivitis is a chronic inflammatory response to the accumulation of supragingival biofilm. Chronic periodontitis is a chronic inflammatory disease that results from a complex polymicrobial infection, leading to tissue destruction as a consequence of the perturbation of the homeostasis between the subgingival microbiota and the host defenses in susceptible individuals (62). Hence gingivitis and periodontitis are a continuum of the same inflammatory disease, prevention of the gingival inflammation by plaque biofilm disruption can prevent the progression of gingivitis into periodontitis in susceptible patients (66).

2.1. Dental Plaque Biofilm

Dental plaque described as the soft, tenacious deposits that develop on the tooth surface which is not readily removed by rinsing with water (67). Also, it adheres on other hard surfaces in the oral cavity such as; restorations, prosthesis including; removable and fixed restorations and dental implants (68, 69). Due to advances in scientific research methods, it is currently recognized as a biofilm (70). According to Socransky and Haffajee (71) and Marsh (72), dental plaque is defined as the diverse community of microorganisms found on the tooth surface as a biofilm, embedded in an extracellular matrix of polymers of host and microbial origin.

Dental plaque can be classified according to its relation to the gingival margin into supragingival and subgingival plaque. Supragingival plaque is located at and above the dentogingival junction and it is most commonly found at the gingival third of the crown of the tooth, interproximal areas, pits, and fissures and also on other such surfaces with irregularities. Subgingival plaque is located below the dentogingival junction and it is usually divided into; tooth adherent zone, epithelial adherent zone and non-adherent zone (73).

2.1.1. Development of Dental Plaque Biofilms

Dental biofilms are formed by an ordered sequence of events, resulting in a structurally and functionally organized, species-rich microbial biofilm (71, 74) (Figure 1). The distinct stages of the dental biofilm formation include:

a. Adsorption of a conditioning film (acquired pellicle) within seconds of tooth eruption or after cleaning, tooth surfaces become coated with a conditioning film of glycoproteins derived mainly from saliva but also from GCF and bacteria. This conditioning film alters the biological and chemical properties of the tooth surface, and the composition of the pellicle directly influences the pattern of subsequent microbial colonization. Microorganisms interact directly with this conditioning film.

b. Reversible adhesion between the cell surface of early colonizers (Streptococcus sp., Actinomyces sp.) and the conditioning film by weak long-range physicochemical interactions. This adhesion allows bacteria to detach quite easily from the surface back to their planktonic state.

c. More permanent attachment involving interactions between specific molecules on the microbial cell surface (adhesins) and complimentary molecules (receptors) present in the conditioning film.

d. Co-adhesion of other strains, in which secondary colonizers adhere to receptors on already attached bacteria, which leads to an increase in the microbial diversity.

e. Multiplication of the attached cells, which leads to an increase in the biomass and synthesis and secretion of exopolysaccharides to form the biofilm matrix (plaque maturation) including the development of microcolonies and water channels to form a large matrix enclosed structure, which facilitates a wide range of intermicrobial interactions (synergistic and antagonistic).

f. The detachment of attached cells to promote colonization in or to another place (74).

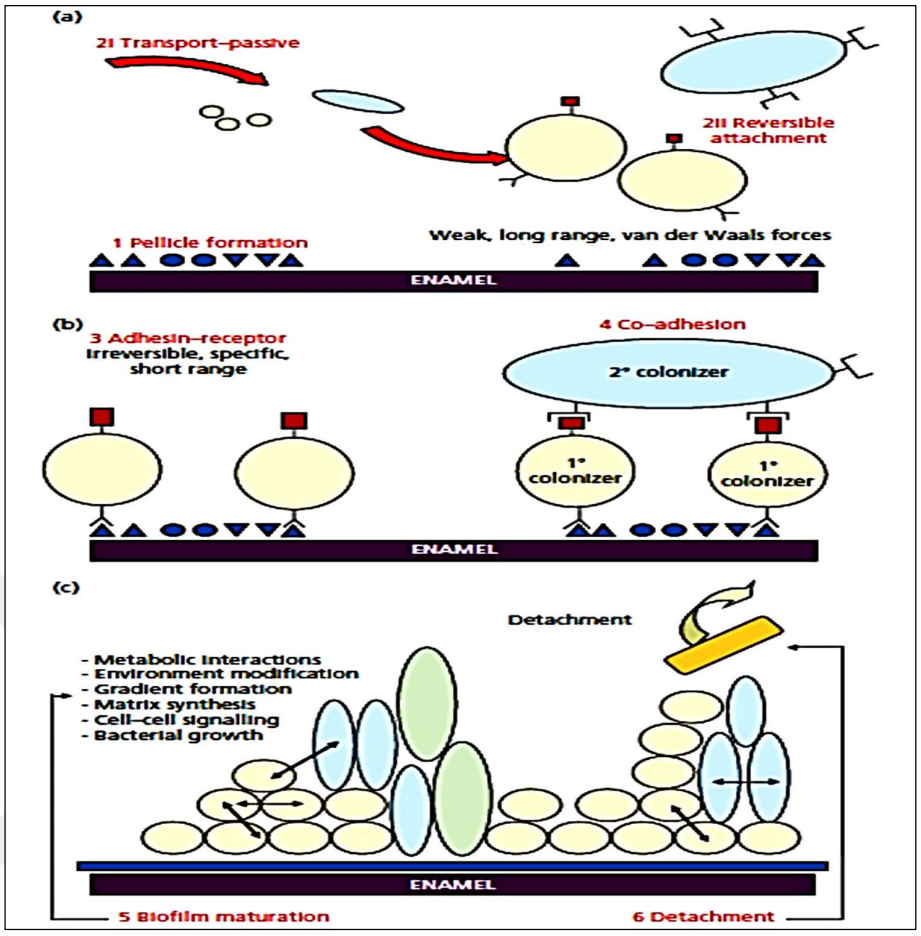


Figure 1. The Stages of Dental Plaque Biofilm Formation (74).

2.1.2. The Structure of Dental Biofilm

Plaque biofilms are complex three-dimensional structures composed of bacterial microcolonies attached to a solid surface like the enamel of the tooth, the surface of the root or dental implants and they are embedded in an exopolysaccharide matrix. Accordingly, the biofilm consisted of 3 components: 1) microcolonies of bacterial cells (15–20% by volume) which is non-randomly distributed in a matrix. 2) voids or water channels between the microcolonies that permit the passage of nutrients and their by-products, 3) exopolysaccharide matrix (EPS) which are the major components of the biofilm (75–80% volume) and produced by the bacteria in the biofilm primarily by using sucrose as a substrate and it maintains the integrity of the biofilm (Figure 2) (71).

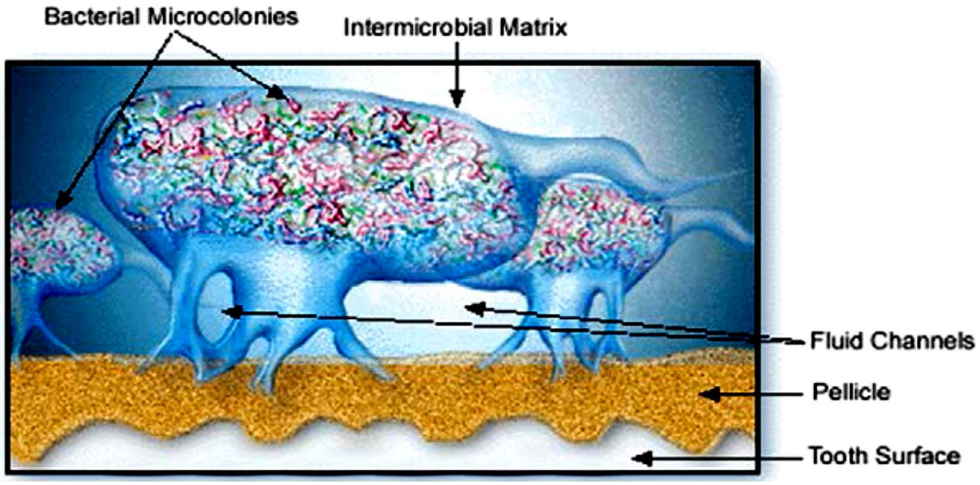


Figure 2. The Graphical Structure of Biofilm (75).

Factors Affecting the Formation and Composition of Biofilm

The composition of the oral microbiota can remain persistent over time as a result of a dynamic balance between the environment and the microflora of biofilm, and this state of balance is known as *microbial homeostasis*. Biofilm composition will shift in response to changes in the local environment and lifestyle (76). Oral environment is subjected to constant transformation depending on the age, the appearance of first teeth, teeth extractions, carious lesions, dentures, fillings, edentulous and transitional changes that may be induced by diet, the variable flow of saliva, and prolonged use of antibiotics (77, 78). Environmental conditions such as temperature, salinity, availability of oxygen, nutrients, variable conditions of pH and redox potential may affect the ecosystem and contribute to changes in species composition of biofilms present in every place. Such changes can disturb biofilm composition and activity, and predispose a site to disease. Therefore, strategies for combating biofilm should be focused on maintaining the healthy composition and activity of these biofilms rather than trying to eliminate them (79).

2.1.4. Ecology of Dental Plaque in Health and Disease

The ecologic plaque hypothesis proposed by Marsh (80) states that changes in the environmental condition leads to ecological shifts which favors the growth of pathogenic bacteria or expression of pathogenic traits. Therefore, both the total amount of dental plaque and the specific microbial composition of plaque may contribute to the progression of periodontitis. In health, the biofilm composition is relatively stable, in a state of dynamic equilibrium or “microbial homeostasis”, and in balance with a steady-state of

low-level immune/inflammatory response. Changes to this steady state may be caused by a nonspecific increase in the accumulation of plaque, or changes in host factors (e.g., changes in hormone levels such as those occurring during pregnancy), or changes in environmental factors (e.g. smoking, stress, diet). These changes result in perturbations of the host response. As inflammation develops in the tissues, GCF flow increases, which may favor the growth of certain species that utilize GCF constituents as a nutrient source. Tissue degradation, increased GCF flow and inflammation can cause a shift in the microbial population, and potentially favoring the growth of the predominantly anaerobic pathogenic species that have been associated with advanced periodontal disease (Figure 3).

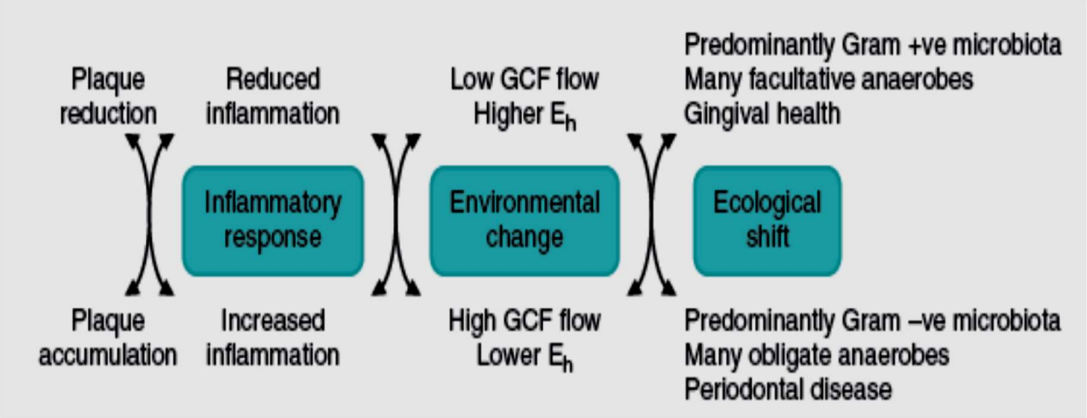


Figure 3. The Ecological Plaque Hypothesis (80).

2.1.5. The Effects of Supragingival Plaque Biofilm

2.1.5.1. The Effects on Gingiva

The classical human experimental gingivitis study by Löe *et al.* (1) clearly proved the direct causal relationship between supragingival plaque and the development of gingivitis. In that study, subjects with clinically healthy gingiva were asked to cease all measures of mechanical plaque control for a period of three weeks. At the end of the three-week experiment of undisturbed supragingival plaque accumulation, the clinical signs of gingivitis (increased GCF flow, gingival swelling, redness and bleeding on probing) developed in all individuals. On the other hand, the reverse was validated by the same group of investigators; gingivitis could be resolved when active tooth brushing was resumed or when the supragingival plaque was removed by an antiseptic e.g. chlorhexidine digluconate and antibiotics (5, 81, 82).

Attström and Egelberg (83) investigated the early histological signs of gingivitis in the animal model. Following microbial supragingival colonization, an inflammatory response was initiated in the clinically healthy gingiva and it was characterized by the migration of neutrophils and macrophages into the junctional epithelium. The migration of neutrophils become more apparent at about 3 days as the plaque flora become more gram-negative.

In humans, clinically detectable gingivitis is observed between 7-21 days and varies among individuals (5). Histologically, the cell types predominating in gingivitis lesion shift from lymphocytes to plasma cells in the extravascular cellular infiltrate as the severity and longevity increase (84).

The composition of microbial flora is slightly varied in healthy gingiva and established gingivitis. Gram (+) cocci and rods are the predominant flora in the healthy state. In established gingivitis, the plaque flora consists of increased numbers of fusobacteria and filamentous organisms as well as spirochetes and spirilla (5). The composition of the flora in chronic gingivitis is non-specific in nature (85).

In general, the extent and severity of gingivitis are directly correlated to the amount of supragingival plaque present (86, 87). Also, the rate of the supragingival plaque accumulation may indicate the susceptibility of the host for periodontal inflammation since supragingival plaque harbors putative periodontal pathogens. However, regular plaque removal prevents the subgingival colonization of these pathogens (88).

2.1.5.2. The Effects on Gingival Crevicular Fluid (GCF)

Gingival crevice fluid (GCF) is an inflammatory exudate around teeth with inflamed gingiva which harbors a complex mixture of substances derived from serum, leukocytes, and structural cells of the periodontium and oral bacteria. These substances possess a great potential for acting as indicators of periodontal disease and healing after therapy (89). Crevicular epithelium may become more permeable with inflammation and GCF is increased in the presence of inflammation (90, 91). It has been reported that the flow of GCF can be detected a few days prior to the evidence of other clinical signs of inflammation. Crevicular fluid appears in altered states of vascular permeability which may accompany gingival inflammation (92). On this basis, its measurement has been proposed as an indicator of periodontal disease activity (89).

Gingival crevicular fluid may be collected by two basic techniques: absorbent filter paper strips and capillary micropipettes. GCF collection methods using filter paper strips may be divided into the intracrevicular and extracrevicular methods. GCF can be collected intracrevicularly through the placement of a filter strip into the gingival sulcus until the resistance is felt. Then, after 3 minutes in place, the strips are removed, dried, and stained with ninhydrin which stains proteins (93). Also, filter paper strip can be placed at the entrance of the crevice for minimal irritation to the gingival sulcus (94). GCF can be collected extracrevicularly when the strips were closely adapted to the buccal surfaces of the teeth, across the gingival margin and onto the attached gingiva. The collection of GCF with capillary pipettes is an unpractical technique for clinical use as it requires collection of relatively large volumes to assure accurate measurement of fluid volume demanding a considerable amount of time for sampling (95). A more precise method is the usage of micropipettes which determines the actual volume of GCF (94).

Measurements of GCF are accomplished by ninhydrin stain assessment or by the use of the periotron. Periotron measures filter strip wetness (impedance) through evaluating the flow of the current. The periotron employs an electronic transducer which measures electrical capacity to estimate GCF, as the isolating properties of the filter paper strip vary according to the quantity of fluid absorbed by the strip. A digital readout registers the area wetted and it is indicative of the volume of fluid collected on the paper strip (96).

The assessment of GCF is either quantitative to assess GCF flow rate and volume changes or qualitative to assess metabolites of bacterial and host origin. Previous investigations have shown that the volume of GCF increases during gingivitis and periodontitis (97). Therefore, the monitoring of the GCF volume has been proposed as a better indicator of gingival inflammation than standard clinical procedures for presence but not the severity of gingival inflammation (98).

2.1.6. Physiological Strategies for the Control of Oral Biofilms

Potential strategies for controlling oral biofilm can be aimed to alter the formation, ecology or structure of the biofilm; such as reducing bacterial adherence, changing the extracellular matrix, altering quorum sensing, regulating the expression of virulence factors, etc. (62).

The following strategies are planned for the control of oral biofilms (99).

1. Control of nutrients by:
   - Addition of base generating nutrients (ex. arginine).
   - Reduction of GCF.
   - Antiinflammatory agents.
   - Inhibition of key microbial enzymes.

2. Control of biofilm through pH:
   - Inhibition of acid production; by using sugar substitutes, antimicrobial agents or fluoride.
   - Stimulation of base production (alkaline); by using arginine, urea or peptides.

3. Control of redox potential by using:
   - Redox agents.
   - Oxygenating agents.

4. Other strategies like:
   - Interfering with communication networks.
   - Preventing the colonization of selected organisms.
   - Dissolving biofilm matrix by enzymes.
   - Replacing pathogen with a less virulent strain.
   - Photoactivation of microorganisms.

2.2. Prevention of Gingivitis

The prevention of gingivitis is based on supragingival plaque control, which may also help to prevent consequent progression to periodontitis in susceptible subjects. Therefore, management of gingivitis is both a primary prevention strategy for periodontitis and a secondary prevention strategy for recurrent periodontitis (100).

Baehni and Takeuchi (7) categorized the prevention of periodontal diseases into three levels depending upon when they are applied as the following:

- Primary prevention: It aims to prevent the development of the disease by protection of individuals from pathogens using barriers between the pathogens and the host. It aims to keep the population in health as it prevents the disease before it occurs.

- Secondary prevention: It aims to cure the disease at an early stage by limiting its progression once the pathogen has contacted the host; trying to arrest the disease and recover health, without damage to the host tissues.
- Tertiary prevention: It aims to arrest or limit the progression of an established disease and to control its negative consequences; trying to restore the host tissues, but with some degree of functional damage.

Based on all of the above, gingivitis can be prevented by:

- Primary prevention that aims to prevent the development of gingivitis based on supragingival biofilm control by means of mechanical and/or chemical self-performed plaque control using different oral hygiene products that are able to limit gingivitis development.
- Secondary and tertiary prevention that aims to treat gingivitis and prevent its recurrence based on arresting the disease progression with proper periodontal therapy that followed by supportive periodontal therapy programs that include both individual (self-performed) biofilm control and periodic re-evaluation with professional plaque control (7).

2.2.1. Primary Prevention

The concept of primary prevention of periodontitis is derived from the assumption that gingivitis is a precursor of periodontitis and the maintenance of healthy gingiva will prevent periodontitis. It is based on supragingival biofilm control by means of mechanical and/or chemical oral hygiene products that are capable to limit gingivitis development. Primary prevention of periodontal diseases includes:

- Educational interventions for periodontal diseases and related risk factors.
- Regular self-performed plaque removal.
- Professional mechanical removal of plaque and calculus.
- Patient motivation and professional oral hygiene instruction (OHI) (7).

For controlling biofilms, various mechanical devices and chemical formulations are designed and developed in order to provide an optimal self-performed oral health and they are referred as "oral hygiene products" (101). Meticulous, self-performed plaque removal can modify both the quantity and composition of supra and subgingival plaque (102). Experiments on animals and human studies have shown that lesions of gingivitis

and chronic periodontitis may resolve following treatment regimens that include the removal of supragingival and subgingival plaque and its mineralized component; calculus (103). It has been demonstrated that following this basic therapy for gingivitis and chronic periodontitis, a recurrent disease can be prevented in most cases and sites in subjects who are enrolled in supportive periodontal therapy programs, including careful professional and self-performed supportive treatment programs to ensure the regular removal of supragingival biofilm (8, 104).

2.3. Treatment of Gingivitis

Periodontal treatment for chronic gingivitis is directed at reduction of oral bacteria and associated calcified and non-calcified deposits (105). In addition, the plaque-retentive factors such as over-contoured crowns, open and/or overhanging margins, narrow embrasure spaces, open contacts, ill-fitting fixed or removable partial dentures, caries, and tooth malposition; must be corrected to restore the gingival health. Also, the modifying systemic factors that may affect treatment and therapeutic outcome (ex. diabetes, pregnancy, etc.) must be controlled. Furthermore, the surgical correction of gingival deformities, as gingival clefts, gingival craters and gingival enlargement that interfere with patients' ability to perform adequate plaque control, may be indicated (106). Treatment of gingivitis is accomplished by supragingival plaque control regimen (SGPC) which involves:

- Professional plaque control (supragingival prophylaxis-SGP)

It includes the removal of dental plaque and calculus accomplished by scaling and polishing procedures using manual, sonic or ultrasonic instruments. The use of chemical agents as an adjunct to professional plaque control may be indicated (105).

- Self-performed plaque control (mechanical and chemical)

Mechanical tooth cleaning through tooth brushing with toothpaste is possibly the most common and the most effective method of oral hygiene practiced by people in the developed countries (107).

A satisfactory response to the treatment is usually attained when personal plaque control measures are performed in conjunction with professional removal of plaque, calculus, and other local contributing factors (108,109). However, patients with chronic gingivitis and without significant calculus, altered gingival morphology and systemic

diseases, may respond to a therapeutic regimen consisting of improved personal plaque control alone (106). Clinical trials indicated that self-performed plaque control programs alone without periodic professional reinforcement, are inconsistent in providing long-term inhibition of gingivitis (9, 110).

- Behaviour modification of patients which could be achieved through:
  - Oral hygiene reinstruction, motivation, and education.
  - Compliance with suggested periodontal maintenance intervals.
  - Counseling on control of risk factors; e.g., diabetes, smoking…etc.

In the management of patients with non-resolving gingival inflammation, treatment may include additional sessions of oral hygiene instruction and education, additional or alternative methods and devices for plaque removal, medical/dental consultation, additional tooth debridement, increasing the frequency of prophylaxis, microbial assessment, and continuous monitoring and evaluation to determine further treatment need. If gingivitis remains following the removal of plaque and other contributing local factors, a comprehensive evaluation of systemic factors (e.g., diabetes, pregnancy, etc.) should be undertaken. If such conditions are existing, gingival health may be achieved once the systemic problem is resolved and plaque control is maintained (106).

Review of several related literature revealed that patient compliance is important for effective plaque biofilm control (102, 111). However, evidence suggests these behavioral changes are not sustained and only effective for a short-term and for this reason effective plaque control remains difficult (112).

2.4. Supragingival Plaque Control

Supragingival plaque control aims to complete plaque removal, which is unrealistic. Ideally, supragingival plaque control should prevent periodontal tissue inflammation and breakdown by reducing the quantity of plaque below an individual's threshold for disease or by changing the quality of plaque to a more tissue-friendly composition. However, as soon as subgingival plaque is established, it cannot be eliminated by using ordinary concepts of oral self-care but requires professional intervention. Consequently, the transition of supra- to a subgingival colonization of plaque formation is crucial for disease progression (10). Supragingival plaque control is considered as the mainstay of primary and secondary prevention of periodontal diseases

and it can be divided into mechanical and chemical, or combination of both approaches (100).

2.4.1. Mechanical Plaque Control

Mechanical plaque control including cleansing of the dentition using traditional toothbrushes with adjunctive manual interproximal aids (dental floss, toothpicks, and the interdental toothbrush) has been shown to be effective in controlling plaque biofilm and maintaining periodontal health (107, 113).

The American Dental Association (ADA) recommends tooth brushing twice and flossing once a day as a regimen for good oral hygiene (114). The challenge is that most patients do not brush in this frequency with adequate time, or use poor tooth brushing technique, and also do not use floss for interdental cleaning, which lead to increased incidence of gingivitis. Additionally, lack of oral biofilm control on other sites like the dorsum of the tongue, mucosal surfaces of the cheek and tonsils as result of inadequate instruction, which serve as reservoir for periopathogens. Thus, even with proper oral hygiene instructions, patients tend to lose the motivation and compliance over time and eventually they return to baseline plaque levels (74).

Adequate mechanical plaque control can not be achieved in some conditions; such as the postsurgical period of oral or periodontal surgery, patients with intermaxillary fixations or with acute mucosal or gingival infections where pain prevents mechanical application and patients with limited dexterities including mentally or physically handicapped patients. For these conditions, the use of chemical agents is compulsory for regular plaque control (74).

Based on the above-mentioned factors, the use of chemical agents in plaque control as an adjunct to/or replacing mechanical plaque control seems logical, especially in populations with a tendency for periodontal tissue inflammation and breakdown (74).

2.4.2. Chemical Plaque Control

Chemical plaque control can be achieved by using various chemotherapeutic agents in different delivery format such as; dentifrices, mouth rinses, sprays etc. as adjunctive to mechanical means. The chemical agents have been proposed for preventing and controlling gingivitis and periodontitis; by inhibiting the formation of a dental biofilm

or controlling the deleterious bacterial by-product. However, they cannot replace the mechanical plaque control (115, 116).

2.4.2.1. Mechanism of Action of the Chemical Plaque Control Agents

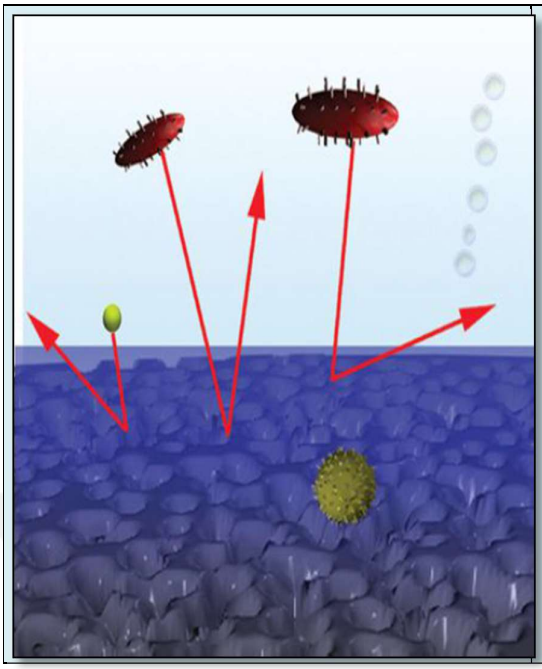
Chemical plaque control may be achieved by different mechanisms of action with a quantitative (reduction of the number of microorganisms) and/or qualitative (altering the vitality of the biofilm) effect as follows (74):

- Preventing bacterial adhesion using *antiadhesives* (Figure 4).
- Inhibiting bacterial growth and/or co-aggregation using *antimicrobials* (Figure 5).
- Eliminating an already established biofilm (disaggregation) using *plaque removal agents* (Figure 6).
- Altering the pathogenicity of the biofilm using *antipathogenic agents* (Figure 7).

2.4.2.2. Categories of Chemotherapeutical agents

Chemical formulations for plaque control were categorized according to their effects by Lang and Newman (117) as follows:

a. **Antimicrobial agents**: chemicals that have bacteriostatic or bactericidal effects *in vitro* and alone cannot show its efficacy against plaque *in vivo*.
b. **Plaque-reducing/inhibitory agents**: chemicals that have quantitative or qualitative effect on the plaque which may or may not be sufficient to prevent the development of gingivitis and/or caries
c. **Antiplaque agents**: chemicals that produce prolong and profound plaque reduction which is sufficient to show benefits in terms of gingivitis and/or caries control.
d. **Antigingivitis agents**: chemicals that reduce gingival inflammation without necessarily effect on the dental plaque, including antiinflammatory drugs.



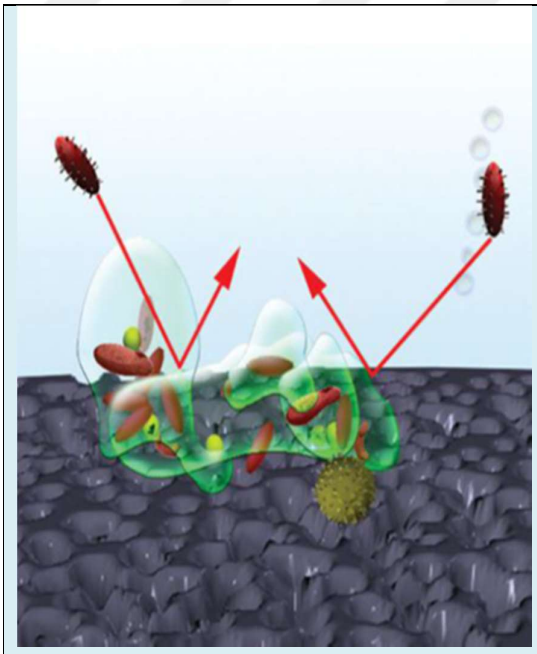
Antiadhesives

Prevention of initial bacterial adhesion to tooth surfaces as the active agent forms a pellicle (blue film) over the tooth surface, interfering with bacterial adhesion (red arrows), thus avoiding bacterial colonization.

They affect the primary plaque-forming bacteria (74).

ex.; amine alcohol, delmopinol which interfere with bacterial matrix formation.

Figure 4. The Mechanism of Action of Antiadhesives (74).



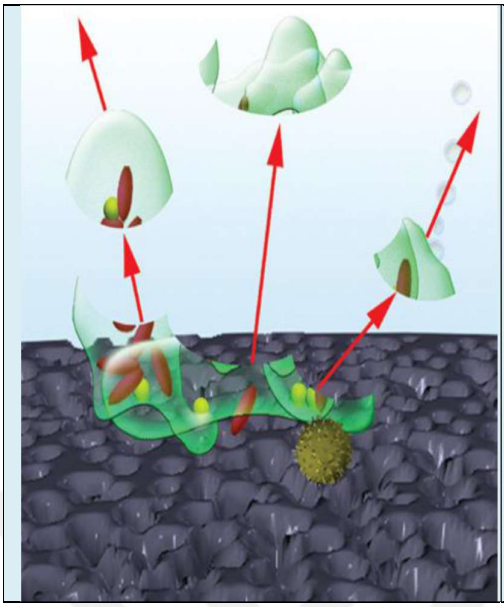
Antimicrobials

Either bactericidal or bacteriostatic alone or combined.

- Inhibition of bacterial proliferation and co-aggregation (bacteriostatic).
- Interference with bacterial division either attaching or already attached bacteria to the tooth surface (bactericidal).

ex.; chlorhexidine, antibiotics (74).

Figure 5. The Mechanism of Action of Antimicrobials (74).



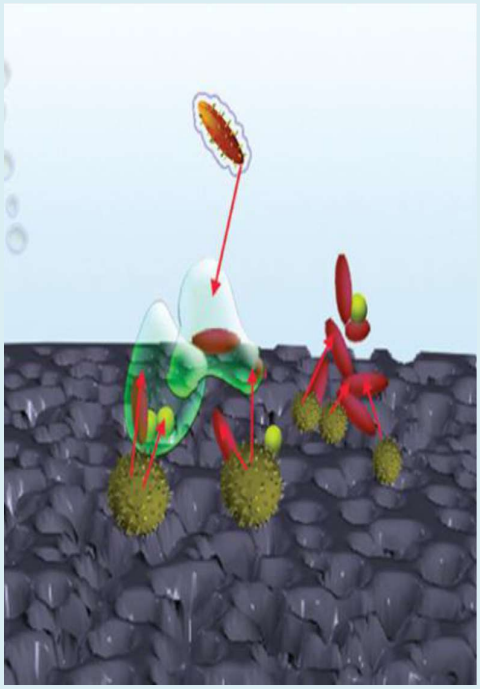
Plaque removal agents
"chemical brushing"

Disruption of biofilm from tooth surfaces by detachment and/or biofilm elimination through:

- Breaking the chemical links between the surface and the biofilms.
- Disrupting the biofilm structure (red arrows).

ex.; hypochlorites, enzymes, amine alcohol (delmopinol) (74).

Figure 6. The Mechanism of Action of Plaque Removing Agents, Chemical Brushing (74).



Antipathogenic agents

Alteration of biofilm pathogenicity without necessarily destroying the microorganisms or enhancement of host immune systems by different mechanisms.

Enhanced host defense systems providing more effective biofilm control by the host (short red arrows).

The presence of defined bacterial species may influence biofilm development and maturation by means of the release of different products such as bacteriocins, or by competition for nutrients (long red arrow).

ex. bacteriostatic antimicrobial agents (74)

Figure 7. The Mechanism of Action of Antipathogenic Agent (74).

According to Mandel in 1988 (116), the antimicrobial agents were subclassified into five general categories as the following:

a. **Antiseptic:** an agent which demonstrates a broad spectrum of antibacterial activity and aiming to kill or prevent the proliferation of all plaque organisms.
b. **Antibiotic:** an agent which is capable of inhibiting or killing a specific group of bacteria.
c. **Enzymes:** they are available as a single or multiple agents, and they can break up or disperse the gel-like matrix which holds the plaque together or modify plaque activity.
d. **Modifying agents:** non-enzymatic, dispersing, denaturing agents that can alter the structure or metabolic activity of bacterial plaque.
e. **Antiadhesives:** agents which interfere with the attachment of all or some of the oral bacteria to the pellicle surface or to each other.

Antiplaque agents have been subdivided by Kornman in 1986 (10) according to their substantivity into three distinct generations:

a. First-generation agents

Antibacterial agents that show very limited substantivity with limited time of action and reduce plaque score by 20-50%, such as; antibiotics, phenolic derivatives, plant extracts, fluorides, quaternary ammonium compounds and oxidizing agents.

b. Second-generation agents

Antibacterial agents that demonstrate good substantivity with prolonged time of action and reduce plaque score by 70-90%, such as; bisbiguanides; CHX is the best example, also triclosan with either copolymer or zinc citrate.

c. Third-generation agents

Agents that interfere or prevent bacterial adhesion with no effect on bacteria, ex: amine alcohols as delmopinol. Products containing sanguinarine, oxygenating agents, saturated pyrimidine and hexetidine also can be included in this group.

Chemotherapeutic agents can be divided according to their individual properties into three categories (12):

a. Group A agents (antiplaque)

They include chemicals with good substantivity and antibacterial spectrum and as well as good antiplaque effects. They can inhibit plaque formation to such an extent that they prevent the development of gingivitis. Therefore, they can be used to replace mechanical cleaning methods for a short time period when it is not possible for the individual to use the mechanical means effectively. They include chlorhexidine, acidified sodium chlorate, salifluor, and delmopinol.

b. Group B agents (plaque-inhibitory)

They include agents with little or no substantivity but with a good antibacterial spectrum. Therefore, they have plaque inhibitory effects but they lack true antiplaque effects. These agents can be used as adjuncts to mechanical plaque control. These chemicals include cetylpyridinium chloride, essential oil and triclosan rinses.

c. Group C agents (low to moderate activity)

They include chemicals or rinses with little or no effect on plaque accumulation and they have limited or no adjuvant effects when combined with mechanical cleaning. They would be expected to have a largely cosmetic role, such as breath-freshening. They include; sanguinarine, oxygenating agents, hexetidine and rinses containing the saturated pyrimidine.

2.4.2.3. Characteristics of Ideal Chemical Agents/ Formulations:

The features of the ideal chemical agent for plaque control have been proposed:

i. It must be specific for chemical plaque control; having antimicrobial capacity against pathogenic flora in both *in vitro* and *in vivo*.
ii. It must be effective: demonstrated meaningful reductions in plaque and gingivitis in both *in vitro* and *in vivo* studies.
iii. It must have higher substantivity *in vivo*.
iv. It must be safe with minimum secondary adverse effects that proved in animal models before the use in humans.
v. It must be stable at room temperature for an extended period of time with no interference between the different ingredients in the formulation.
vi. It must have acceptable taste and cost with the ease of use (74).

2.4.2.4. The Rules of American Dental Association for Mouth Rinses and Dentifrices

Chemotherapeutic agents have been developed in order to assist in the control of plaque and gingivitis. In 1985 the Council on Scientific Affairs of ADA established guidelines for the acceptance of antigingivitis and/or antiplaque agents. They evaluated the safety and usefulness of dental products based on the results of biological, laboratory, and/or clinical studies. These guidelines have been revised in 1997, 2011 and states that:

"Examples of products evaluated under these guidelines include mouth rinses and toothpastes containing agents that would: destroy, inhibit or modify plaque; including its pathogenicity for gingivitis and microbiologic growth in general, those that modify the attachment of plaque microorganisms to their natural sites and those that act by other antimicrobial mechanisms to reduce or prevent gingivitis".

Any chemotherapeutic agent to be accepted as an effective agent for the treatment of gingivitis, by ADA Council on Dental Therapeutics, must reduce or modify plaque and demonstrate effective reduction of gingival inflammation over a period of at least 6 months. The agent must also be safe; non-toxic, non-mutagenic, non-carcinogenic, does not induce adverse effects on oral soft and hard tissues and does not affect the oral flora (118).

2.4.2.5. Classical Methods to Test Oral Antiseptics

In order to assess the plaque-inhibitory and antiplaque activity of chemical agents, successive phases of evaluation have been proposed and different designs and models are used (119) as follows:

2.4.2.5.1. *In vitro* Studies

- Bacterial tests used to evaluate the antimicrobial activity of a product by measuring the minimum inhibitory concentration, the minimum bactericidal concentration and time-kill assay (suspension test) against different bacterial species.
- Bioavailability and activity can be assessed by different chemical methodologies such as spectrophotometry or by indirect methods such as staining.
- Biofilm models allow formulations to be tested *in vitro* against sessile biofilm bacterial cells, which may better simulate real-life conditions. Various and different *in vitro*

biofilm models have been proposed, but no standardized and accepted model is available (71, 74, 119).

2.4.2.5.2. *In vivo* Studies

2.4.2.5.2.1. Antimicrobial Tests *in vivo*

The antimicrobial activity of chemical agents can be tested by measuring the amount and duration in the reduction of salivary bacteria following a single exposure to a product which is a useful predictor of substantivity (120). Additionally, obtained data can be a useful predictor of plaque inhibition: agents showing the greatest and longest suppression of salivary bacteria are usually the most effective ones (121). A crossover design is used in this test with a placebo and a positive control (119).

2.4.2.5.2.2. *In vivo* Biofilm Study Models

These study models evaluate the effects of different formulations on disks of enamel, dentin or other materials inserted into the mouth of patients with different prosthetic devices and then retrieved for the evaluation of the biofilms formed in their presence. Crossover designs are used with this model (74).

2.4.2.5.2.3. Eight-hour Substantivity Study

This study model is a short-term test described by Bonesvoll *et al.* (122, 123) to determine whether or not and if, for how long a formulation performs a persisting effect *in vivo*. The failure of a formulation to show substantivity would prove an inappropriate effect on the inhibition of plaque development. 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 the pre-existing plaque, plaque and salivary bacteria are studied for the following eight hours while the participants cease oral hygiene measures. A crossover design, with a placebo and a positive control, is used to test all agents in the same individuals (119).

2.4.2.5.2.4. Plaque Regrowth Model Studies

This study model was first proposed and most frequently performed by Addy *et al* (124). It is aimed to evaluate the plaque-inhibitory effect of a formulation *in vivo* while any other oral hygiene measures are stopped during the test phase. Primarily plaque-free

teeth undergo a four-day period with no oral hygiene measures except the rinsing with the allocated formulation. It verifies whether or not a mouth rinse *per se* is able to suppress plaque development and to what extent. If no plaque inhibition has observed, no further effect of the rinsing solution can be expected when oral hygiene is performed (125). A crossover approach (with a placebo and a positive control) is chosen for this study (119).

2.4.2.5.2.5. Experimental Gingivitis Model Studies

Experimental gingivitis study is a short-term study that conducted in parallel design over three weeks test. It was first performed by Löe *et al*. (1) and clearly proved the etiological role of plaque in the development of gingivitis. It was implemented to investigate the influence of an agent on the development of plaque and gingivitis in the absence of mechanical oral hygiene. In this model, the ability of a formulation to inhibit gingivitis and plaque is assessed weekly. Once a tested agent has shown its potential to inhibit plaque this model clarifies whether the plaque inhibiting effect also affects the development of gingivitis (119).

2.4.2.5.2.6. Home-use Study

Home-use studies are long-term studies that performed in parallel design to test the efficacy of antiplaque and antigingivitis 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 protocol includes rinsing and mechanical oral hygiene (119). 0.2% CHX cannot be used in this study model since it is not designed for long-term application (126). Council of Dental Therapeutics in 1986 (127) have proposed characteristics of these trials for validation of the results; they must be double blind (patients and examiner), controlled (negative and/or positive controls), minimum duration of 6 months and accompanied by a microbiologic evaluation to assess the overgrowth of pathogenic, opportunistic or resistant strains. Microbiologic sampling and evaluation of plaque and gingival indices should be carried out at least at baseline, the final evaluation, and an intermediate point (3 months).

2.4.2.6. Chemical Agents for Plaque Control

2.4.2.6.1. Active Agents

2.4.2.6.1.1. Antibiotics

Antibiotics are antimicrobial substances capable of inhibiting or killing of specific microorganisms by causing leakage of cellular contents or affecting microbial metabolism. Different groups of antibiotics have demonstrated an efficacy on dental biofilm with retained serum and GCF levels such as penicillins, tetracyclines, metronidazole, vancomycin, kanamycin, and spiramycin. Despite the evidence of the efficacy of local administration of certain antibiotics in gingivitis, antibiotics are not accepted to be used routinely as supragingival agents because of the potential risk of the development of resistant organisms, patient hypersensitivity, and superinfection by fungal organisms (128, 129).

2.4.2.6.1.2. Enzymes

Enzymes have two groups based on their mechanisms of action for plaque inhibition. The first group involves dextranase, mutanase, proteases, and lipases. They disrupt dental biofilm by interfering with bacterial attachment or disintegrating existing plaque. The second group includes glucose oxidase and amyloglucosidase. They act by enhancing the host defense mechanisms by salivary lactoperoxidase system converting thiocyanate to hypothiocyanite. The hypothiocyanite possesses inhibitory effects upon oral bacteria, particularly streptococci, by interfering with their metabolism. Limited scientific evidence available and their *in vivo* effect on gingivitis has been contradictory as well as no long-term studies are available (74).

2.4.2.6.1.3. Amine Alcohols

Delmopinol and octapinol are surface active agents with limited antimicrobial activity *in vitro* and *in vivo* (130, 131). They act by inhibiting the biofilm matrix formation or disruption of the biofilm matrix, accordingly reducing bacterial adherence. Additionally, delmopinol inhibits glucan synthesis by *S. mutans* and reduces acid synthesis by bacteria. Their use is limited due to their adverse effects such as dental staining, temporary feeling of mucosal numbness and burning sensation (74).

2.4.2.6.1.4. Detergents

Detergents are common ingredients in toothpaste and mouth rinse products added for their foaming effect and surfactant activity that reduce the surface tension and create the impression of cleanliness. The most significant and frequently used detergent or surfactant is sodium lauryl sulfate (SLS). SLS has a limited antimicrobial and plaque-inhibitory effect (123, 132). SLS inhibits the growth of a number of microorganisms as it adsorbs and penetrates through the porous cell wall, it interacts with components of the cell membrane; lipids and proteins. It leads to an increase in cell permeability of the bacteria, which may result in leakage of intracellular components and cell lysis (133). SLS has a limited usage only in dentifrice formulations since its use has been associated with oral hypersensitivity reactions, including cheilitis, stomatitis or aphthous ulcers, burning sensation and desquamation. These negative effects result from the elimination of protective mucin layer by SLS (134).

2.4.2.6.1.5. Metal Salts

Zinc is antibacterial agent that added in mouth rinses and toothpaste formulations as zinc chloride, zinc citrate, zinc sulfate, zinc acetate….). Zinc ions (Zn^{+2}) have the ability to limit bacterial growth, inhibit plaque formation and the glycolytic sequence in oral anaerobic bacteria, and to restrict the bacterial ability to convert urea to ammonia. Also, they can inhibit some bacterial enzymes (41, 42). Zinc has broad spectrum antimicrobial properties against different bacteria such as *S. mutans* (42). Zinc is retained in dental plaque and inhibits its regrowth without disrupting the oral ecology (41). Also they can reduce the bacterial colonization to the tooth surfaces (49). Zinc ions (Zn^{+2}) exhibit anti-VSC effects since zinc ion has two positive charges which result in an affinity for sulfur and thereby forming inert zinc sulfides with low solubility and reducing the expression of the VSCs (135). Several clinical studies demonstrated that zinc salts ranging in concentration from 0.2-2.0%, alone or in combination with antimicrobial agent (eg. triclosan, sanguerine, hexetidine, CPC or CHX), were effective in reducing calculus formation, controlling halitosis, gingivitis and inhibiting plaque formation (41- 43, 136, 137). Its oral use in high concentrations has been limited due to its astringency and unpleasant taste (138).

2.4.2.6.1.6. Oxygenating Agents

Sodium peroxyborate and peroxycarbonate, and hydrogen peroxide (H_2O_2) are oxygenating agents that produce their antimicrobial effect by releasing oxygen. No long-term data are available for peroxyborate and peroxycarbonate, and only one study for H_2O_2 has been published which demonstrated significant benefits in terms of the modified gingival index in 6 months (74, 139).

2.4.2.6.1.7. Stannous Fluoride

Stannous fluoride has been incorporated in dentifrices, mouth rinses, and gels since 1940. But, it is not frequently formulated in mouth rinses due to its limited stability in aqueous solution (74). In a systematic review, the 0.454% stannous fluoride formulation provided significant benefits in terms of gingivitis reduction but its use is limited due to dental staining (74, 140). A combination of stannous fluoride and amine fluoride has demonstrated increased bactericidal activity with 8 hours of action (74).

2.4.2.6.1.8. Other Fluorides

Sodium fluoride and sodium monofluorophosphate are found in most dentifrices and they are useful in reducing caries incidence with no plaque-inhibitory or antiplaque properties (74).

2.4.2.6.2. Natural Products

2.4.2.6.2.1. Herbal Products

For many years, natural products such as herbs and plant extracts have been used in oral hygiene products as sanguinarine extract and other herbal ingredients such as; chamomile, echinacea, sage, clove, myrrh, rhatany, peppermint oil, tea tree oil, meswak, aloe vera, turmeric, neem, green tea, propolis, and xylitol. (17, 141, 142). They have antibacterial and plaque inhibitory properties by suppressing the growth of bacterial strains and enzyme activity (143, 144). However, limited data is available. Sanguinarine exhibites a moderate antiplaque efficacy with no antigingivitis effect. Also, the use of sanguinarine is associated with oral leukoplakia (74).

2.4.2.6.2.2. Essential Oils

Essential oils (EO) are phenolic compounds that have been used as antiseptics and disinfectants for a long time (145). A fixed blend of thymol, eucalyptol, methyl salicylate, benzoic acid and boric acid termed "essential oils" in alcohol. It has multiple and complex mechanisms of action such as antibacterial, plaque inhibitory and antiinflammatory actions (146, 147). It can penetrate plaque biofilm and exert its bactericidal activity resulting in a concomitant decrease in plaque mass and pathogenicity (148).

Its antibacterial action is based on its concentration; at high concentration, it leads to cell wall disruption and protein precipitation. Whereas, at low concentration, it causes inhibition of bacterial enzymes and reducing glycolysis. Its plaque inhibition is achieved by reducing bacterial adherence and its antiinflammatory effect is based on its antioxidant activity (143, 144). EO may be equivalent to CHX for long-term control of gingival inflammation but not better at plaque reduction than CHX in short and long-term studies (149). It has adverse effects including a burning sensation and tooth staining. Also, it has affected by a controversy concerning the association of alcohol-containing mouth rinses and oral cancer. However, critical assessment of the literature does not support an association (74).

2.4.2.6.2.3. Triclosan

Triclosan is a non-ionic bisphenolic with a broad-spectrum antibacterial activity and plaque-inhibitory effect (143, 144, 150). It interferes with plaque metabolism and disrupts the bacterial cell wall. It may also induce antiinflammatory effects through inhibition of the cyclooxygenase and lipoxygenase pathways, which reduces the synthesis of prostaglandins and leukotrienes. In dentifrice formulations, when triclosan combined with copolymer, zinc citrate or pyrophosphate, the antimicrobial activity and substantivity improved to 8 hours. There are no relevant adverse effects, but a risk of carcinogenic product formation (chloroform) was suggested in an *in vitro* study testing the combination of triclosan and free chlorine present in water (74).

2.4.2.6.2.4. Bisbiguanides

Several bisbiguanide antiseptics; including CHX, alexidine, and octenidine; possess antiplaque activity by binding to cell membranes and ability to kill a wide range of microorganisms by damaging the bacterial cell wall. They act by altering the integrity

of bacterial cell membrane. CHX gluconate is the most widely studied bisbiguanide for plaque inhibition and the prevention of gingivitis. It is a cationic bisbiguanide with a single chlorine substitute in each phenol ring. CHX is considered as the gold standard antiplaque and antigingivitis agents. It acts as an antimicrobial agent causing cell wall damage as well as plaque inhibition by binding to cell membranes (12, 13, 74).

2.4.2.6.2.5. Quaternary Ammonium Compounds

Benzylconium chloride and cetylpyridinium chloride (CPC) are monocationic surface-active agents which are capable of reducing surface tension, adsorbing to negatively charged surfaces and disrupting bacterial cell membranes. CPC has a broad antimicrobial spectrum induces rapid killing of gram-positive pathogens and yeast in particular. CPC has a moderate plaque-inhibitory activity that may be correlated to its low substantivity of only 3-5 hours resulted from its rapid desorption, loss of activity and less retention (123, 151). Therefore, CPC has an equivalent antibacterial activity to CHX but with less plaque inhibitory effect and gingivitis reduction (121, 123). Also, the associated adverse effects of CPC are less frequent than that of CHX; such as tooth and tongue staining, transient gingival irritation, and aphthous ulcers (74).

2.4.2.6.2.6. Hexetidine

Hexetidine is a pyrimidine derivative with some antimicrobial activities against Gram-positive and Gram-negative bacteria and yeast *in vitro*. However, *in vivo* results have not demonstrated plaque-inhibitory or antiplaque activity due to a limited oral retention and antimicrobial activity not lasted more than 90 minutes (74).

2.4.2.6.2.7. Povidone Iodine

When iodine is combined with the synthetic polymer povidone, it demonstrated an antibacterial activity with very limited plaque inhibitory action as a result of limited substantivity for only 1 hour at 1% concentration of this combination (74).

2.4.2.6.3. Other Evaluated Products

Some products such as; acidified sodium chlorite (ASC), ClO_2, salifluor, polyhexamethylene biguanide hydrochloride and herbal products are tested for of plaque inhibition and revealed promising results. However, they have to be further assessed for antiplaque and plaque-inhibitory effects (74).

2.4.2.7. Delivery Format for Chemotherapeutic Agents

Chemotherapeutic agents available for chemical plaque control are delivered in different formats such as rinses, gels, dentifrices, chewing gums, aerosols or sprays, varnishes, sustained release devices, lozenges and irrigators (74).

A mouthwash or mouth rinse is defined as a non-sterile aqueous solution used mostly for its deodorizing, refreshing or antiseptic effect and it is designed to reduce oral bacteria, remove food particles, and reduce bad breath and to provide a pleasant taste. They are generally classified as either cosmetic or therapeutic or a combination of these. Cosmetic rinses are commercial or over the counter products; that help to remove oral debris before or after brushing, suppress bad breath, diminish bacteria in the mouth and refresh the mouth with a pleasant taste. Therapeutic rinses often have the benefits of their cosmetic counterparts, but also contain an added active ingredient, ex; fluoride or CHX, that helps to protect against oral diseases (49). Mouth rinsing is reported to be favored by the public because of its ease of use and breath-freshening effect (152). More recently, it is recognized that the level of mechanical oral hygiene practice is inadequate despite technological innovations. Therefore, the use of antimicrobial mouth rinses for controlling plaque and gingivitis is recommended. Mouth rinses consist of a mixture containing the active component, usually an antimicrobial component, water and/or ethanol as solvent, surfactants, humectants, flavoring agent, sweeteners, coloring agents, and preservatives (49, 153). Mouth rinses contain one or more therapeutic agents as the active ingredient such as antimicrobial, antiplaque, antiinflammatory, anticalculus, anticaries, healing enhancing, antihypersensitivity, and antihalitosis agents (49)

2.5. Chlorhexidine

Chlorhexidine is a synthetic antimicrobial drug which has been widely used as a broad spectrum antiseptic in clinical and veterinary medicine since 1953. It was first introduced in human use as an antiseptic cream for skin wound in 1954 (12, 154). CHX is available in various forms such as digluconate, acetate and hydrochloride salts which are less soluble in water. The first usage of CHX gluconate in dental practice was in washing operation sites and disinfecting root canals (155). Then in 1969, Schroeder proved the antiplaque activity of CHX (156).

Chlorhexidine has broad-spectrum antimicrobial activity and it is effective *in vitro* against both Gram (+) and Gram (-) bacteria, including aerobes and anaerobes as well as

yeasts, fungi and viruses, including human immunodeficiency virus (HIV) and hepatitis B virus (12). CHX is bacteriostatic at low concentration, whereas it is bactericidal at high concentration (13). Its antibacterial action is due to an increase in cellular membrane permeability followed by precipitation of the cytoplasm and coagulation of the cytoplasmic macromolecules. It has also been shown that CHX can reduce the adherence of *Porphyromonas gingivalis* to epithelial cells. This effect is probably due to the binding of CHX to the bacterial outer membrane and therefore it may have the same effect on the adherence of other plaque bacteria (12).

Chlorhexidine has a symmetrical molecule consisting of four chlorophenyl rings and two biguanide groups connected by a central hexamethylene bridge (126). CHX is a strong base and it is di-cationic at pH levels >3.5, with the two positive charges on either side of the hexamethylene bridge (157). One charged end of CHX molecule binds to the tooth surface, whereas the other remaining interact with the bacterial membrane as microorganism approaches the tooth surface that recognized as *a pin cushion effect* (13). It reversibly and tightly binds to tooth structure, oral tissues, and dental plaque and releases slowly over time resulting in 8-12 hours of sustained antimicrobial activity (123). Its superior antiplaque activity is the result of its substantivity and pin-cushion effect (124).

The primary mechanism of action of CHX involves membrane disruption, causing concentration-dependent growth inhibition and bacterial cell death. The cationic nature of CHX enables it to bind to tooth surfaces and oral mucosa, reducing pellicle formation and resulting in persistent effect over a period of time which referred as substantivity thereby reducing bacterial viability and inhibiting plaque growth (158). Substantivity is influenced by the concentration and the pH of the drug, temperature, and the length of the contact time of the solution with the oral structures (123).

Animal experiments with radiolabelled CHX have shown that the half-life of CHX is 4 days and the primary route of excretion is through the feces with minimal metabolic changes and up to date, no evidence of carcinogenic substance formation or tetragenic alterations has been reported following long-term use (12). Additionally, CHX has very low toxicity because it is poorly absorbed by the gastrointestinal tract (GIT). Even that if some solution is unintentionally swallowed, CHX does not penetrate oral epithelium but it initially binds to the mucosal surfaces of the GIT. Therefore, the mode of action of

CHX when used as mouth rinse is purely topical. Systemic toxicity, microbial resistance and superinfection do not occur with the oral use of CHX (154).

2.5.1. Adverse Effects of Chlorhexidine Usage

Even though CHX is not toxic, it has an unpleasant taste, alters taste sensation and produces brown staining on the teeth and restorations which is very difficult to remove. Staining can also affect the mucous membranes and the tongue and may be related to the precipitation of chromogenic dietary factors on the teeth and mucous membranes. It is probable that one cationic group attaches CHX to the tooth or mucosal surface, while the other cationic group produces the bactericidal effect of damaging the bacterial cell wall. However, this cationic group can also attach dietary factors such as gallic acid derivatives (polyphenols) found in some foods and many beverages, including tea, coffee, and tannins from wine to the molecule and hence to the tooth surface (207). Also increased supragingival calculus formation, mucosal irritation and parotid swelling have been reported (128, 159).

2.5.2. Clinical Efficacy of Chlorhexidine as Antiplaque and Antigingivitis Agent

In 1970, Löe and Schiött (160) showed that a complete inhibition of plaque and prevention of gingivitis could be achieved by twice daily rinsing with 10 ml of 0.2% CHX solution (20 mg dose) in an experimental gingivitis study. The antiplaque and antigingivitis effects of CHX were then confirmed in both short-term and long-term human clinical trials, and in animal studies (128, 161-167). CHX is still considered as the gold standard for chemical plaque control (13).

2.5.3. Clinical Indications of Chlorhexidine

Chlorhexidine is available in many forms such as mouth rinses, gels, chips, sprays, toothpaste, varnishes and chewing gum. The most commonly used form of CHX is the mouth rinse with a concentration of 0.1–0.2% (74). Based on the clinical situation, the duration of product usage and the main objective of the intervention, many different indications for using CHX have been proposed as follows:

2.5.3.1. Single Use

Chlorhexidine is used once immediately as preoperative rinse or irrigation to reduce bacteremia, bacterial load in the oral cavity and aerosol contamination associated with sonic and ultrasonic devices (74).

2.5.3.2. Short-term Use

Chlorhexidine is most frequently used for a short-term period either for prevention of biofilm formation or for the therapeutic aim. CHX may be indicated as an adjunct to mechanical plaque control for the prevention of biofilm formation after periodontal treatment and also in patients with intermaxillary fixation, acute mucosal and gingival infections; or for therapeutic aim in order to control pathogenic microorganisms in patients with necrotizing gingivitis, candidiasis, peri-implant mucositis, peri-implantitis, and periodontitis. CHX can be used as the only oral hygiene care in the postsurgical period to prevent postoperative infections. In periodontal treatment, CHX may also be as an adjunct to periodontal debridement in full mouth disinfection using different formulations as mouth rinses, irrigators, and sprays for the tonsils and gels for the tongue dorsum (74).

2.5.3.3. Long-term Use

Chlorhexidine can be indicated for long-term use in order to prevent biofilm formation in some conditions in which mechanical plaque control is deteriorated such as patients with: periodontitis, fixed or removable orthodontic appliances, gingival enlargement or overgrowth, dental implants and a disability. Also, CHX may be indicated for long-term use to prevent oral and systemic complications in patients with blood dyscrasia or immunosuppression who are at high risk to develop oral infections. In addition, CHX is used for the prevention of other oral conditions like chemo/radiation-induced oral mucositis in patients with head and neck cancer, caries, candidiasis, recurrent aphthous ulcers and in therapy and secondary prevention of halitosis (74).

2.6. Aloe Vera

Aloes are perennial succulents or xerophytes, which develops water storage tissue in the leaves to survive in dry areas of low or erratic rainfall. Aloe plant is one of the ancient medicinal plants which used for its healing properties. *Aloe barbadensis*,

commonly named as Aloe vera (ALV), is the most commercialized aloe species and processing of the leaf pulp has become a large worldwide industry (Figure 8). It used in cosmetic, pharmaceutical, health and food products. The commercial products are made from processed leaves (168). The aloe leaf can be divided into two major parts; outer green rind including the vascular bundles and the inner colorless parenchymal pulp containing the aloe gel (19).

Figure 8. Aloe Vera Plant and Aloe Leaf (169).

Four major types of processed products are identified: whole leaf extract; decolorized whole leaf extract; inner-leaf gel; and dried bitter latex. Decolorization removes pigments and anthraquinones (laxative) from the whole leaf extract. The ALV whole leaf extract is also referred to as "whole leaf Aloe vera juice", Aloe juice or non-decolorized whole leaf extract and it contains both the gel from the inner parenchyma leaf pulp and the latex. The inner leaf liquid material should be referred to as “gel”. Also, interchangeable terms were found in the literature for the “gel” as inner pulp, mucilage tissue, mucilaginous gel or jelly, inner gel, and leaf parenchyma tissue gel (168).

The raw pulp of ALV contains approximately 98.5% water, while the mucilage or gel consists of about 99.5% water. The remaining 0.5 – 1.0% of solid material consists of more than 200 compounds and over 75 of these compounds have biological properties including water-soluble and fat-soluble vitamins, minerals, enzymes, polysaccharides, phenolic compounds (anthraquinones), organic acids and compounds such as salicylic acids, saponins, sugars, lignin, and amino acids (Table 1). The bulk of aloe leaf is made of mainly anthraquinones and polysaccharides as 62.3% and 57.6% of the dry weight of the rind and pulp (168). Many of the medicinal effects of Aloe leaf extracts have been attributed to the polysaccharides. However, the therapeutic effects have not been correlated well with each individual component. In fact, it is believed that these biological

activities should be assigned to a synergistic action of the compounds contained therein rather than a single chemical substance (19, 168).

Many researchers have identified partially acetylated mannan (or acemannan) as the primary polysaccharide of the gel, while others found pectic substance as the primary polysaccharide. It has been hypothesized that this heterogeneous composition of the ALV pulp may contribute to the diverse pharmacological and therapeutic activities which have been observed for aloe gel products (19). The physical and chemical constituents of the products derived from ALV plants differ depending on the source (e.g. part of the plant), the species of the plant, the climate conditions, seasonal and grower influences, and processing techniques (168, 171).

Table 1. The Chemical Composition of Aloe Vera Leaf Pulp and Exudate in Summary (19, 171).

Constituents	Number and identification	Properties and activity
Amino acids	Provides 20 of the 22 required amino acids and 7 of the 8 essential ones	Basic building blocks of proteins in the body and muscle tissues
Anthraquinones (Phenols)	Provides Aloe emodin, Aloetic acid, alovin, anthracine, Aloe-emodin, aloetic acid, anthranol, aloin A and B (barbaloin), isobarbaloin, emodin, ester of cinnamic acid	Analgesic, antibacterial
Enzymes	Anthranol, barbaloin, chrysophanic acid, smodin, ethereal oil, ester of cinnamonic acid, isobarbaloin, resistannol	Antifungal and antiviral activity but toxic at high concentrations
Hormones	Auxins and gibberellins	Wound healing and antiinflammatory
Minerals	Calcium, chromium, copper, iron, manganese, potassium, sodium and zinc	Essential for good health and enzyme activity
Salicyclic acid	Aspirin like compounds	Analgesic
Saponins	Glycosides	Cleansing and antiseptic
Steroids	Cholesterol, campesterol, lupeol, sistosterol	Antiinflammatory agents, lupeol has Antiseptic and analgesic properties
Sugars	Monosaccharides: Glucose and Fructose Polysaccharides: Glucomannans/polymannose	Antiviral, immune modulating activity of acemannan
Vitamins	A, B, C, E, choline, B12, folic acid	Antioxidant (A, C, E), neutralises free radicals

2.6.1. Mechanisms of Action

It has been claimed that the polysaccharides in aloe vera gel have therapeutic properties including immunostimulation, antiinflammatory effects, wound healing, promotion of radiation damage repair, antibacterial, antiviral, antifungal, antidiabetic and antineoplastic activities, and stimulation of hematopoiesis and antioxidant effects (19, 172).

2.6.1.1. Wound healing Effects

Several mechanisms have been proposed for the wound healing effects of aloe gel, which include keeping the wound moist, increase epithelial cell migration, more rapid maturation of collagen and reduction in inflammation (19). Glucomannan; mannose-rich polysaccharide, and gibberellin (growth hormone) interact with growth factor receptors on the fibroblast stimulating its activity and proliferation, which in turns significantly increases collagen synthesis (173).

2.6.1.2. Antimicrobial Activities

Aloe vera gel exhibited strong bactericidal activity against both cariogenic and periodontopathic bacteria (52). Also, the ALV gel is reported to be virucidal against Herpes simplex, Herpes zoster, varicella- zoster, influenza virus, and pseudorabies viruses (174). Various percentages of ALV gels have demonstrated bactericidal and fungicidal activity against the following organisms in culture media: *S. aureus*, *Streptococcus viridans*, *C. albicans*, *Corynebacterium xerosis,* and the five strains of *S. mutans* that most commonly found in dental plaque (51). This activity was attributed to a number of pharmacologically active compounds including anthraquinones, aloin, aloe-emodin, aloetic acid, anthracine, aloe mannan, aloeride, antranol, chrysophanic acid, resistanol, and saponin (174).

2.6.1.3. Antiinflammatory Activities

Aloe vera gel appears to exert its antiinflammatory activity through bradykinase activity, and thromboxane B2 and prostaglandin E2 inhibition (175). ALV inhibits the cyclooxygenase pathway and reduces prostaglandin E2 production from arachidonic acid or reduces the leukocyte adhesion and tumor necrosis factor-alpha level (TNF-α) (19). Also, it inhibits polymorphonuclear leucocyte (PMN) infiltration, bradykinin activity and

histamine formation by magnesium lactate (170). The anthraquinones (aloin, aloe-emodin, e.g.), the aloe sterol; including campesterol, β-sitosterol, lupeol, and cholesterol; the peptidase bradykinase, and C-glucosyl chromone have been reported to be responsible for its antiinflammatory effect (175).

2.6.1.4. Immunomodulatory Effects

The polysaccharides in Aloe gel demonstrated direct immunomodulating activities by the activation of macrophage cells to generate nitric oxide and secreting cytokines as TNF-α, interleukins (IL-1, IL-6) and interferon-γ (INF-γ), presenting cell surface markers and enhancing phagocytosis. Also, they increase the number of circulating monocytes and macrophages and activate the complement by the alternative pathway (171). In addition, alprogen inhibits calcium influx into mast cells, thereby inhibiting the antigen-antibody-mediated release of histamine and leukotriene from mast cells (176).

2.6.1.5. Antioxidant Effects

Aloe vera contains very strong antioxidant nutrients such as glutathione peroxidize activity, superoxide dismutase enzymes and a phenolic antioxidants, which are responsible for its antioxidant effects (19). Recent reports demonstrated an antioxidant action for some constituents of ALV gel. Three aloesin derivatives from aloe (namely isorabaichromone, feruoylaloesin, and p-coumaroylaloesin) exhibit potent free radical and superoxide anion-scavenging activities. Also, these aloesin compounds inhibited cyclooxygenase-2 (COX-2) and thromboxane TxA2 synthase; which can partially explain the healing effects of ALV (177).

2.6.1.6. Moisturizing and Antiaging Effect

Moisturizing effects of ALV appear due to water and polysaccharide components creating a jelly-like consistency which holds the water within the mix and minimizes its evaporation when applied to drying tissues. Also, ALV has humectants properties which retain moisture in the tissues (178). Aloe stimulates fibroblast which produces the collagen and elastin fibers making skin more elastic and less wrinkled (179). Although the carcinogenicity of whole leaf extract of ALV was reported in experimental animals after oral administration in 2-year study in mice, there is inadequate evidence in humans for the carcinogenic ity of ALV (168).

2.6.2. Uses of Aloe Vera

Aloe vera has been incorporated in many oral products such as; mouth rinses, toothpastes, tooth gels, topical gels, and oral sprays. It can be used in the treatment of many oral diseases and conditions such as acute oral lesions; viral infections as herpes simplex, herpes zoster, aphthous ulcers, and fungal infections like candidiasis, denture stomatitis, and thrush. It can be directly applied to periodontal surgical sites, extraction sockets, and mechanically or chemically traumatized gingival tissues. Also, ALV can be used in many chronic oral diseases such as lichen planus and benign pemphigus, gingival lesions associated with AIDS and leukemia, migratory glossitis, geographic tongue and burning mouth syndrome. ALV can also be used around dental implants to control inflammation from bacterial contamination (180).

2.6.3. Studies Evaluating the Efficacy of Aloe Vera on Dental Plaque and Gingival Inflammation

Villalobos *et al.* (21) evaluated the effect of 50% ALV containing mouth rinse on plaque and gingival inflammation by a randomized, clinical study with a parallel design in 40 subjects with chronic gingivitis. ALV and placebo mouth rinses were used twice a day following tooth brushing for a period of 30 days. Plaque index (PI) and gingival index (GI) were evaluated at baseline, day 15, and day 30. Results revealed significant reductions in plaque and gingivitis scores after 30 days usage of 50% ALV mouth rinse with tooth brushing. It was concluded that ALV at 50% concentration could reduce plaque and gingival inflammation.

Upasna and Sujal (22) conducted a randomized, parallel grouped and double-blind clinical trial to evaluate the efficacy of 98% ALV on reduction of plaque and gingivitis scores in comparison to 0.2% CHX and 0.22% tea tree oil mouth rinses in 30 subjects over a period of 21 days. The subjects were instructed to rinse with 10 ml mouth rinse twice daily, after breakfast and after dinner, for 1 minute without tooth brushing. PI and GI evaluated at baseline and on day 21. All mouth rinses yielded a significant reductions in PI scores from baseline to day 21 ($p<0.05$). However, in terms of GI, a significant differences were observed in ALV and CHX groups from baseline to day 21 ($P< 0.05$).

Chandrahas *et al.* (23) performed a randomized, parallel-grouped and double blind clinical trial to evaluate the antiplaque and antigingivitis efficacy of 100% ALV on the

experimental gingivitis model in comparison to 0.2% CHX and placebo. One week prior to the commencement of the study, all participants underwent to professional cleaning and instructed to continue their regular oral hygiene. During the first two weeks of the study (from day 0 to day 14), all participants were instructed to wear the plaque guard on the premolar and molar regions during every tooth brushing. In the morning of day 15, all participants began to rinse with 10 ml of the allocated mouth rinse for 1-minute twice daily, with no food and/or drink intake for 2 hours. PI, GI and bleeding index (BI) assessed on day 0, 14 and 22. The results demonstrated significant reductions in PI, GI, and BI scores after the rinse regimen began in both ALV and CHX compared with placebo ($p<0.05$). ALV showed significant reduction of PI and GI but it was less than CHX ($p<0.05$). It was concluded that ALV mouth rinse could be an effective antiplaque agent. Also, with appropriate refinements in taste and shelf life, it could be an affordable herbal substitute for CHX.

Karim *et al.* (24) performed a randomized, parallel-grouped and triple blind clinical trial to evaluate the clinical efficacy of 99% ALV mouth rinse for the inhibition of plaque accumulation and gingival inflammation in comparison to CHX and distilled water (DW) in 345 healthy dental students. The subjects were asked to rinse with 15 ml of the allocated mouth rinse twice daily for one minute for 30 days. PI and GI were assessed at days 0, 15 and 30. ALV and CHX mouth rinses similarly reduced PI and GI scores at days 15 and 30 without statistically significant difference between them ($p>0.05$).

Gupta RK *et al*. (25) conducted a randomized, parallel-grouped and double blind clinical trial to evaluate the antiplaque efficacy of 100% ALV mouth rinse on a 4-day plaque regrowth model in comparison to 0.2% CHX and saline water. Subjects were asked to rinse with 10 ml of the allocated mouth rinse twice daily for one minute for 4 days, after breakfast and lunch as the only daily oral hygiene care. After 4 days, there were significant reductions in PI scores in ALV and CHX groups ($p<0.05$). CHX group demonstrated the maximum reduction of PI compared to ALV, but this reduction was not statistically significant ($p>0.05$).

Chhina *et al*. (26) performed a randomized, single blind, placebo-controlled study with a parallel design to evaluate the clinical efficacy of 99.6% ALV mouth rinse in comparison to 0.2% CHX and placebo rinse on a 4-day plaque regrowth model. 90 healthy subjects were instructed to rinse with 10 ml of the solution twice a day for 1

minute. No eating or drinking allowed before 2 hours of rinsing. On the 5th day, CHX and ALV groups presented similar plaque scores which were significantly lower than placebo. Evaluation of the adverse effects in ALV group revealed taste disturbance in 4% of subjects with no staining after 4 days of usage. In CHX group, staining, taste disturbance and burning sensation were observed in 40%, 25%, and 2% of subjects respectively. No adverse effects were reported in the placebo group. It was concluded, that ALV mouth rinse had a comparable antiplaque efficacy to 0.2% CHX mouth rinse and it could be considered as a feasible alternative.

Vangipuram *et al.* (27) conducted a randomized, double blind, placebo-controlled study with a parallel design to evaluate the clinical efficacy of 99% ALV mouth rinse on periodontal health in comparison to 0.12% CHX and placebo over a period of 30 days. A total of 390 subjects were asked to brush their teeth twice daily with a standard toothbrush and toothpaste (without an antiplaque agent) and to rinse with 10 ml of the allocated mouth rinse twice daily for 1 minute over a period of 30 days. PI and GI evaluated at baseline, day 15, and day 30. Significant reductions in the mean PI and GI scores were observed in ALV and CHX groups ($p<0.05$) with no significant difference between them ($p>0.05$). It was concluded that ALV mouth rinse is equally effective to 0.12% CHX in reducing plaque and gingivitis. ALV could provide better preventive home care therapy.

Daing *et al.* (28) conducted a randomized, double blind, placebo-controlled study with a three arm crossover design in 15 healthy subjects to evaluate the plaque inhibitory effect of 98% ALV mouth rinse in a four day *de novo* plaque accumulation model in comparison to 0.2% CHX and DW. A washout period of 10 days was established between the treatments to exclude the carryover effect of the experimental mouth rinse. Subjects were instructed to rinse twice a day for a minute with 10 ml of their assigned rinse. PI was scored and Plaque area (PA) was analyzed using digital standardized orthoradial photography and computer-based calculation. After four days of *de novo* plaque formation, the distribution of mean PI and PA of three mouth rinses was statistically significant in favor of CHX ($p<0.05$). ALV showed intermediate values at PI and PA whereas the negative control showed the highest values.

Although the results of the previous studies are promising, limited research is available to support the recommendation of ALV containing mouth rinse over other conventional antiseptic mouth rinses for chemical plaque control (29, 30).

2.7. Chlorine Dioxide

Chlorine dioxide (ClO_2) is a synthetic, oxyhalogen compound with known antimicrobial properties that widely used in industry for disinfection and control of bacterial biofouling. It is also used to control taste and odor; for oxidation of metal ions and color removal in other applications (181). Since it is approved by the FDA and EPA as a disinfectant, a sterilizer, and an antimicrobial agent, its use increases in different industries including the dairy, beverage and food to control microbiologic growth, and for the removal of biofilm; as well as deodorizing and bleaching agent. Also, it is used in municipal water supplies and paper pulp industry (15). It is known as an oxidizing biocide that kills microorganisms by disruption of the nutrient transport across the cell wall (40). The oxidative consumption of critical biomolecules; proteins, nucleic acids, unsaturated fatty acids, and polysaccharides; by ClO_2 is primarily responsible for its wide range of biocidal activity, and its single electron reduction product (ClO_2^-) can also act as a reactive oxidant toward many electron-donating biomolecules (e.g. methionine, pyruvate, urate, and endogenous thiols such as cysteine) (39). In dentistry, ClO_2 containing products demonstrated their ability to oxidatively consume volatile sulfur compounds (VSCs) which are responsible for halitosis, elevate the oxygen tension in both saliva and plaque, remove residual organic solutes, and suppress the activity of bacterial proteolytic enzymes (181).

Chemically, the ClO_2 molecule is composed of one atom of chlorine and two atoms of oxygen. ClO_2 has an uneven number of chlorine atoms and unpaired electron, therefore it may be considered as a free radical (Figure 9). The chemical structure of ClO_2 is pH-dependent (182, 183).

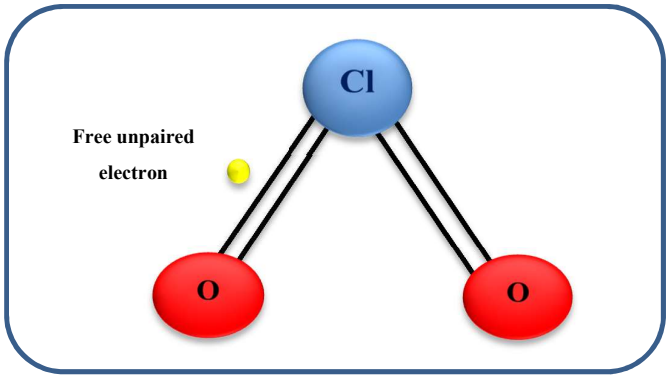


Figure 9. The Molecular Structure of ClO_2.

Chlorine dioxide is available as gas and as a dissolved gas in a solution. At equal concentrations, gaseous ClO_2 is more effective than aqueous ClO_2 as it offers greater penetration into small spaces where the aqueous form cannot reach (184). Chlorine dioxide exists in the water as gaseous ClO_2 (little or no dissociation) and thus is able to permeate through bacterial cell membranes and destroy bacterial cells (185).

ClO_2 is commonly generated from the chlorite ion by acidification; mainly as sodium chlorite buffered to a pH around 7 to 8 and above. Because instability of ClO_2 gas at room temperature or with sunlight exposure, and short shelf life of an hour, the oxidizing and germicidal capabilities of ClO_2 must be saved by activating ClO_2 just prior to use or prolonging its stability through a stabilization process. Stabilized ClO_2 is a buffered solution of sodium chlorite and ClO_2 is retained in the solution as a labile complex (reactive) when the solution is alkaline. Once the solution becomes acidic with pH below 3, ClO_2 is released (186).

Despite ClO_2 is a strong oxidizing agent and a particularly fast disinfectant, there are no reports in the scientific literature regarding its toxicity that caused by skin contact or ingestion, or its mutagenicity. At high concentration, ClO_2 causes rapid bacterial and viral kill by softening and destroying the cell wall or viral capsid. In contrast, human cells are apparently unaffected since they do not have similar cell walls. In addition, human skin and body are likely protected from the general oxidative effects of ClO_2 by many reducing agents in the cells and blood, such as catalase, glutathione, superoxide dismutase, vitamins E, C, A, B complex, uric acid, zinc, and selenium. This is probably the same internal protective mechanism that prevents damage from oxygen and free radicals. However, bacteria and viruses do not contain most of these reducing compounds (187).

2.7.1. Mechanism of Action of Chlorine Dioxide

The specific mechanism of action of ClO_2 on cells has been debated for many years (188). Early research supposed that cell death was due to the disruption of protein synthesis by ClO_2 (189). Later, the inhibition of the total dehydrogenase enzymes, protein synthesis and DNA of bacteria was proposed (190). Then, the cell membrane was reported as the primary target of ClO_2 (184). The mode of action of ClO_2 involves the disruption of cell protein synthesis and membrane permeability control (191). Damage to the genetic materials is also a possibility. ClO_2 reacts with the amino acids and RNA within the cell,

prevents the production of proteins, affects the cell membrane by switching membrane proteins and fats; and prevents inhalation (186). Finally, it was concluded that the antimicrobial activity of ClO_2 was achieved by direct chemical degradation of cellular material or deactivation of critical enzyme system on the cell wall with its ability as an oxidizing agent to penetrate the cell wall and disrupt the metabolic pathways (188).

2.7.1.1. Antimicrobial Action

Chlorine dioxide has a variety of antimicrobial actions; bacteriostatic, bactericidal, fungistatic, fungicidal, viralistatic, viricidal, algicidal and sporicidal effect under optimal conditions of pH and concentration (33, 40, 183). When the pH is lower than neutral, molecular ClO_2 releases from the aqueous solution and ClO_2 reacts as an antimicrobial agent. The oxygen atom first binds to a single atom (the one being oxidized) and then is dissociated from chlorine. An electron is then given up to chlorine forming the chloride ion. The subsequent liberation of molecular oxygen ($O^{\bullet}$) which is a potent oxidizing agent for protein, nucleic acid, lipids, and polysaccharides. This mechanism results in bactericidal and bacteriostatic effects on the microbial ecology of aerobic, facultative and anaerobic pathogenic bacteria (183).

Chlorine dioxide exhibits bactericidal effect at low concentration (ppm) on *S. mutans, S. Sangius*, *P. gingivalis*, *Bacteroid Melaninogenius, A. actinomycetumcommitans, C. albicans* (35). ClO_2 has also fungicidal and viricidal effect by damaging the fungal cell membrane and viral capsid resulting in cell lysis (192, 193).

Furthermore, ClO_2 can act as bacteriostatic agent by inhibition of the bacterial growth of anaerobic species through different mechanisms; raising the level of oxygen in saliva and plaque matrix, disruption of the nutrient transport across cell membrane and oxidation of the nutrient substrates through bacterial enzymes inhibition. Inhibition of glycosyltransferase enzyme results in prevention of the degradation of sucrose into glucose and fructose, subsequently retarding the formation of dextrans, glucans, lucans, and levans, thereby interfering with the nutrient supply to the bacteria and reducing number of *S.sangius* (35). Additionally, ClO_2 has fungistatic and viralistatic effects that achieved by interruption of the life cycle of fungi and inactivating the virus genome (194).

2.7.1.2. Effect on Plaque Formation

Chlorine dioxide affects the process of plaque formation through subsequent steps of actions on acquired pellicle formation, bacterial aggregation, and the plaque growth; which alters the sequence of formation process such as bacterial adhesion (195).

At the beginning, ClO_2 inhibits the process of acquired pellicle formation by oxidizing the sulfated glycoprotein in the pellicle. This inhibition alters the sequence of formation process and subsequent steps are retarded. Also, ClO_2 prevents the bacterial aggregation by oxidizing the sulfide bond of biochemical compounds like glycosaminoglycan (GAG), proteoglycan, glycoprotein, sugars, proteins and lipids and forming sulfate (SO_2), which can also affect bacterial growth. Furthermore, ClO_2 retards the plaque growth by inhibition of bacterial agglutination and by degradation/oxidation of the plaque mass through the oxidation of bacterial by-products, nutrients, and debris from dead and dying cells ex; carbohydrates, chondroitin sulfate, GAG, glucoprotein, proteins and lipids which are of salivary origin and essential for bacterial agglutination and the growth of the plaque mass (195).

2.7.1.3. Inhibition of the Volatile Sulphur Compounds and Subsequent Reduction of Gingival Inflammation

Inhibition of the VSCs; hydrogen sulfide (H_2S), methyl mercaptan and dimethyl sulfide, is achieved by oxidizing the sulfide bond of sulfur-containing amino acids; the precursors of VSCs. This results reduction of oral malodor and reduction of tissue permeability to VSCs and consequently inhibits the penetration of pathogenic by-product from plaque; such as endotoxins to gingival tissue and inhibits subsequent inflammation (196).

2.7.1.4. Healing Enhancement

Chlorine dioxide, as a strong oxidizing agent and a free radical, can quickly neutralize reactive molecules such as cytokines and oxygen free-radicals. These substances are produced in the body by macrophages in response to stress or infection which cause inflammation and pain. Also, other potential irritants found in wounds are similarly oxidized or reduced, such as leukotrienes, TNFs and ILs. Unlike iodine compounds or CHX, healing is not retarded by ClO_2 (187). ClO_2 increases the integrity

of epithelium and reduces the number of plasma cells as a consequence of the addition of oxygen. Also, it eliminates the cellular and food debris from the wound area (197196).

2.7.2. Available Formulation/Composition of Chlorine Dioxide Mouth Rinse

Chlorine dioxide is available in different delivery systems such as; mouth rinses, mouth sprays, dentifrices, gels, irrigation solutions, chewing gums, lozenges, dry powders, and dental implements such as dental floss and tape. It is used in the prevention and treatment of oral diseases and conditions such as; oral malodour, plaque, gingivitis, periodontitis, herpetic lesions, oral infections that may develop following dental procedures (osseous surgery, tooth extraction, periodontal flap surgery, dental implants and scaling and root planning), dentoalveolar infections (cellulitis, osteomyelitis), acute necrotizing ulcerative gingivitis, infectious stomatitis, and cancrum oris (Noma), either in humans or pets by topical application to gingival and mucosal tissues or teeth surfaces (196).

There are a variety of formulations and compositions of ClO_2 mouth rinse available for use. All oral compositions contain the chlorite ion, mainly as sodium chlorite, as the main active ingredient for generating the optimum amount of ClO_2 and its concentration differ from one composition to another with similar pH of >7.5, optimally at a range of 8-12. Stabilized ClO_2 is available in a single container system as single phase composition. Whereas, active ClO_2 is available as dual Phase System and is provided either in double container system or in the same single container but with two compartments. One container contains an unactivated solution of chlorite ions (sodium chlorite) as the first phase while the other container contains the inactivated solution consists of oxidizer and pH adjuster (buffer as phosphate) and the other ingredients with no chlorite as the second phase. These ingredients must be compatible with each other and with the active agent (ClO_2). Amount of two solutions are added to each other to form the activated solution of ClO_2. Both compositions are available either as a single or combination formula (331). The chlorite ion may be combined with one or more of such agents in a single delivery system to provide combined effectiveness. For instance, a combination of ClO_2 and zinc, a combination of ClO_2 and fluoride (195).

2.7.3. *In vitro* and *in vivo* Evaluations of the Antimicrobial Efficacy of Chlorine Dioxide

Grootveld *et al*. (40) conducted a controlled study *in vivo* to investigate the efficacy of ClO_2 containing mouth rinse on the salivary levels of *S. mutans*, Lactobacilli and *C. albicans* in a group of 33 elderly patients for a period of 14 days with 10 dental students as a control group. 20 ml of the mouth rinse used by the test group 3 times a day after twice-daily brushing while mineral water rinse was used by the control group. Salivary samples were collected from the participants at the baseline and after 14 days. The results demonstrated a marked reduction in salivary levels of *S.mutans* and Lactobacilli in test group whereas no significant reduction was observed for *C. Albicans.* It was concluded that 0.1% ClO_2 containing mouth rinse had bactericidal activity *in vivo* as it suppressed the salivary levels of cariogenic bacteria; *S.mutans* and Lactobacilli.

Albayaty *et al*. (56) performed an *in vitro* study to investigate the antimicrobial action of ClO_2 gel and hyaluronate gel (Gengigel®) on dental biofilm and on selected bacteria representing dental biofilm. Pooled supra and subgingival dental biofilms were obtained from healthy individuals and incubated aerobically and anaerobically. Although positive results were obtained from both agents against the tested microorganisms, antibacterial action with a larger diameter of inhibition zones was produced by the ClO_2 gel. Under SEM, ClO_2 gel produced obvious alterations in the bacterial morphology whereas hyaluronate gel demonstrated no changes. It was concluded that ClO_2 gel demonstrated stronger and obvious antibacterial activity against dental biofilm and bacteria compared to hyaluronate gel. Consequently, it was suggested that ClO_2 gel can be proposed as a good alternative ingredient for the development of professional gel for controlling and inhibiting various types of dental biofilm and microorganisms.

Drake and Villhauer (57) performed a bacterial assay study to compare the bactericidal activity of a stabilized ClO_2 oral rinse to several other commercially available oral rinses. Oral bacteria associated with oral disease evaluated in this study included: *A. viscosus, Actinomyces naeslundii, Streptococcus oralis, S. mutans, Enterococcus faecalis, P. gingivalis***,** *S. sanguinis, Peptostreptococcus micros, Actinomyces odontolyticus, Prevotella nigrescens, Aggregatibacter actinomycetemcomitans*. The results of this study revealed that the stabilized ClO_2 oral rinse demonstrated strong

bactericidal activity against oral bacteria that are associated with periodontal and endodontic infections as well as dental caries and staphylococcal infections.

LaRue (55) evaluated the efficacy of ClO_2 against biofilm phenotype bacteria with the standard zone of inhibition (ZOI) methods and a hydroxyapatite-coated tooth model accompanied with four assays in comparison to 0.12% CHX, 0.07% CPC, EO, and 0.2% delmopinol. The results of ZOI, SEM analysis, CFU, optical density assay and antibiofilm activity demonstrated that ClO_2 had a comparable activity, but not superior to the other tested solutions. However, ClO_2 demonstrated higher inhibitory activity against *C. albicans* than other solutions.

Herczegh *et al.* (58) examined the antibacterial properties of sodium hypochlorite (NaOCl), CHX, EO and high purity ClO_2 (Solumium, ClO_2) on selected common oral pathogen microorganisms and on dental biofilm *in vitro*. Antimicrobial activity of oral antiseptics was compared to phenol. The investigated oral pathogens were *S. mutans, L. acidophilus, A. actinomycetemcomitans, E. faecalis, V. alcalescens, E. corrodens,* and *C. albicans*. Dental plaque samples were collected from the upper first molars of healthy young students. The biofilm disrupting effect of antiseptics was measured after dissolving the crystal violet stain from biofilm by the photometer. The results showed that hyper-pure ClO_2 solution was more effective than other currently used disinfectants in reduction of aerobic bacteria and Candida yeast. The biofilm dissolving effect of hyper-pure ClO_2 was found significantly stronger than CHX and EO after 5 min treatment. It was concluded that hyper-pure ClO_2 has a potent disinfectant efficacy on oral pathogenic microorganisms and a powerful biofilm dissolving effect compared to the current antiseptics. Therefore, high purity ClO_2 suggested as a new promising preventive and therapeutic adjuvant for home oral care and in dental or oral surgery practice.

2.7.4. Studies Evaluating the Antiplaque and Antigingivitis Efficacy of Chlorine Dioxide

Goultschin *et al.* (36) conducted a pilot, double blind study with crossover design to test the efficacy of a metastable chlorous acid/chlorine dioxide (MECA) formulation on developing plaque and salivary bacterial count in a group of 18 volunteers aged between 20-27 years over periods of 33 days. Two different formulations of mouth rinse: high concentration (0.16% sodium chloride in an activating system), low concentration (0.04% sodium chloride, comparably activated) and placebo mouth rinse (activating

system alone) were used as the only means of oral hygiene for a 5-day period with 9 days washout period. The high and low concentration groups showed 34.5% and 13.5% reduction of dental plaque scores, respectively, compared to the placebo group where there is no any significant change in the number of salivary bacteria.

Yates *et al*. (37) conducted a randomized, double blind study with 5-cell crossover design to assess the substantivity of 3 acidified sodium chlorite (ASC) mouth rinses as well as to evaluate the plaque inhibitory effect of the same ASC mouth rinses in 4-day plaque regrowth model in comparison with 0.12% CHX and placebo control. The 3 ASC and CHX mouth rinses produced similar reductions in salivary bacterial counts at 7 hours compared to the placebo. At 30 and 60 minutes, significantly greater reductions were produced by 2 ASC rinses compared to the CHX rinse. Plaque indices were significantly lower in the ASC and CHX groups compared to the placebo with no significant differences between plaque scores for the 3 ASC rinses and CHX. It was concluded that ASC mouth rinses possessed potent plaque inhibitory effect similar to CHX when used as the only oral hygiene measure and that possibly gained from a persistence of antimicrobial action in the mouth.

Paraskevas *et al*. (15) performed a randomized examiner-masked, two-group parallel experiment to investigate the plaque inhibitory effect of 0.01% active ClO_2 mouth rinse in comparison to 0.2% (CHX) mouth rinse in a 3-day plaque accumulation model in 77 healthy subjects. The plaque level was assessed at baseline and the fourth day accompanied by the evaluation of compliance and VAS questionnaire. ClO_2 mouth rinse was found to be a less potent plaque inhibitor than CHX. However, participants preferred the taste of the ClO_2 mouth rinse and experienced less taste alteration compared to CHX.

Mueller (38) evaluated the antiplaque and antigingivitis effect and clinical safety of ClO_2 (acidified chlorite) containing toothpaste over a 3-week period in a randomized, double blind, parallel study including 14 subjects without moderate or advanced periodontitis. The experimental group received two dispensers dentifrice containing 0.6% chlorite (pH 4.9) and 0.06% sodium fluoride whereas the positive control group received dentifrice containing 0.3% triclosan and 0.24% sodium fluoride. Subjects were instructed to dispense equal quantities of each part onto the toothbrush and brush twice daily with dispensed dentifrice for 60 sec and then evaluated after 3 weeks. A significant reduction in PI ($p< 0.0482$) in both groups with no significant differences regarding GI and BOP.

Also, no adverse effect was observed or reported during the study period. Obviously, ClO_2 toothpaste demonstrated clinical safety and efficacy in reductıon of dental plaque accumulation, gingivitis, and bleeding on probing.

Yadav *et al.* (39) conducted a randomized, triple blinded, crossover microbiological clinical trial to evaluate the efficacy of 0.1% stabilized ClO_2 and 0.2% CHX containing mouth rinses on the inhibition of tongue coat accumulation and dental plaque formation using a 4-day plaque regrowth model with 10 days of washout period in 25 healthy dental students. Subjects were asked to refrain from oral hygiene and to only rinse with allocated mouth rinse for 1 minute under supervision twice a day for 4 days. Bacterial samples were taken from the buccal mucosa and tooth surface at baseline, 4 hours post rinsing and at the fourth day of each period. Plaque scores, the extent of tongue coating, and the wet weight of tongue coat in gram were measured at baseline and at the fourth day of each period. There was a significant reduction in CFU in ClO_2 and CHX after 4 hours from the first rinse. In conclusion, the plaque inhibitory effect, rate of tongue coat accumulation, and antibacterial property of ClO_2 mouth rinse is comparable to the 0.2% CHX gluconate mouth rinse.

Yeturu *et al.* (16) conducted a randomized, single blind, parallel group, controlled trial among 90 subjects undergoing orthodontic treatment to evaluate the efficacy of three mouth rinses containing ALV, CHX, or ClO_2 against plaque and gingivitis over a period of 15 days. Plaque and gingivitis were assessed by PI, GI respectively at baseline and at follow-up after 15 days. Subjects were instructed to rinse with 10 ml of mouth rinse for 1 min, twice daily for 15 days. There were significant reductions in mean plaque and gingival scores in all the 3 groups at follow-up compared to baseline higher reductions in PI and GI were found in CHX group compared to ALV group. However, no significant differences were observed between CHX and ClO_2 with respect to mean plaque and gingival score reduction. Findings of this study suggested that ClO_2 can be a suitable and economical alternative for CHX.

Despite the limited number and heterogeneity of the studies on the antiplaque action of ClO_2, ClO_2-based oral products represents an effective alternative in term of preventive or other therapeutic measures for shielding against or combating periodontal diseases; and maintaining high level of oral hygiene.

2.8. Xylitol

Xylitol is a naturally occurring nonfermentable five-carbon sugar alcohol derived from fruit, vegetables, and berries and it is artificially manufactured from xylan-rich plant materials. It is approved as a natural sweetener by US FDA and American Academy of Pediatric Dentistry. XYL is available in many forms such as; chewing gums, gummy bear snacks, syrups, mouth rinses and dentifrices. Habitual consumption of 5–7 g of XYL, at least three times a day, decreases the incidence of dental caries by increasing the salivary flow and pH and reducing the number of cariogenic (*S. mutans*) and periodontopathic (*Helicobacter pylori*) bacteria, dental plaque, xerostomia, gingival inflammation and erosion of teeth. Although XYL consumption reduces *S .mutans* count in dental plaque, it has no effect on microbial composition of dental plaque or saliva in general. It reduces the adhesion of these microorganisms to teeth surfaces and reduces their acid production potential (44).

Xylitol demonstrated clear inhibitory effect on the formation of the experimental biofilms of six bacterial species (*S. mutans, Streptococcus sobrinus, Lactobacillus rhamnosus, A. viscosus, P. gingivalis and Fusobacterium nucleatum*) on hydroxyapatite discs (HA). Therefore, XYL is not only efficient in inhibiting the acid production of cariogenic bacteria, but also in preventing the formation of a multispecies biofilm. This results confirm the relevance of the use of XYL for the prevention of dental plaque-related oral diseases (58).

A 4-day plaque regrowth study compared the inhibitory effect of XYL-containing preparation or solution at different concentrations with or without 0.2%CHX on *de novo* plaque formation in 10 periodontally healthy subjects with 10 days of washout. The subjects were instructed to chew or suck 2 XYL combined gums or candies a time, twice a day, or to rinse with 10 ml of DW or XYL solution twice a day for 1 minute each and all mechanical tooth cleaning were refrained. A statistical significant difference was observed between DW and 5% XYL or 20% XYL ($p<0.05$) as well as between DW and XYL+CHX ($p<0.01$). No statistical significant difference was observed between XYL-containing chewing gum or candy and DW ($p>0.05$). In conclusion, the combination of higher percentage of XYL solution with CHX may effective for preventing periodontal diseases and carious lesions (45). The combination of CHX and XYL was reported to be more effective against *S. mutans* and *S. sangius* than CHX or XYL alone. It was found

that *S. sanguis* was more sensitive to the antiseptic effects of CHX alone, while *S. mutans* colonies were more sensitive to the XYL/CHX solution. This synergistic effect of XYL has been reported when added to probiotic and CPC. However, limited studies are available in the literature on the synergistic effects of XYL and other oral products (44, 45).

2.9. Essential oils of Citrus and Mint

Essential oils have been incorporated in mouth rinses for their ability to break the cell walls of bacteria, leading to cell lysis, to inhibit their enzymatic activity, and to kill the microorganisms. Furthermore, they prevent bacterial aggregation and slow their reproduction (198). Also, they have been suggested as antioxidants and preservatives in food (45). The mechanisms of action of EOs are dependent on their chemical composition and the location of one or more functional groups on the molecules present in them (199). Essential oils such as; citrus oils and mint oils have been added to oral care products as a flavoring agent, but they also demonstrated antibacterial and antioxidant activities (45). Their bactericidal effect on *S. mutans* has been reported (47, 48).

Antibacterial evaluation of spearmint EO (Menta Spicata) against *S. mutans* and *S. pyogenes* by gas chromatography and GC-mass spectrometry demonstrated that spearmint EO was effective against *S. pyogenes* and *S. mutans* and could significantly slow down their multiplication (48).

A combination of peppermint, spearmint, and almond oils displayed antibacterial activity against early, intermediate, and late plaque colonizers including; *S. sanguis*, *S. oralis, S. gordonii, A. naeslundii, F. nucleatum, A. actinomycetemcomitans*, and *P. gingivalis* strains 381 and W83 with *S. aureus* as a non-oral control in a spectrophotometric assessment of inhibition of planktonic growth and a growth inhibition zone assay. It was effective against Gram - and Gram + oral bacteria with the highest efficacy against Gram - species. This explains the beneficial clinical effects of the oils in reducing periodontal inflammation (60).

In vitro evaluation of the antimicrobial effects of lemon EO (*Citrus Limonum*) and bitter orange EO (*Citrus aurantium)* on multi-species biofilms demonstrated potent microbial reductions that were not only similar to those of 1% NaOCl but even higher than those achieved by 0.2% CHX, in some cases. It was found that *C. aurantium* EO and NaOCl were the most effective solutions and inhibited the growth of all microorganisms.

Whereas *C. limonum* EO promoted 100% reduction of *C. albicans* and *E. coli*, and 49.3% of *E. faecalis* biofilms. On the other hand, 0.2% CHX did not achieve the complete elimination of any microorganism, and was less effective against *C. albicans* and *E. coli*, with a reduction of 68.8% and 86.7%, respectively. Lemon EO showed antifungal potential against three *Candida* species; *C. albicans*, *Candida tropicalis*, and *Candida glabrata*) (61).

Considerable number of studies were performed to investigate the antimicrobial action of each agent separately; ALV, ClO_2, Zn^{+2}, XYL, and citrus and mint oils in *vitro* and *in vivo* and they demonstrated a promising plaque inhibitory effect. However, a natural mouth rinse containing a combination of these agents has to be evaluated for its possible plaque inhibitory effect and to the extent of our knowledge, no plaque regrowth study has been conducted on this combination mouth rinse. Thus, this study was planned to assess the plaque inhibitory effect of this combination when it used as the only oral hygiene measure in individuals who refrained from mechanical plaque control in a 4-day plaque regrowth model.

The hypothesis of the present study was a combination mouth rinse, containing ALV, ClO_2, Zn^{+2}, XYL, and CMO, is capable of inhibiting supragingival plaque formation and gingival inflammation *in vivo* and it could be an alternative to CHX, the gold standard for chemical plaque control.

3. MATERIAL AND METHODS

3.1. Subject Selection

Study participants were volunteer dental students of Yeditepe University, Faculty of Dentistry in the time period between October/2016-April/2017. They were examined using dental mirror[1], explorer[2], tweezer[3] and periodontal probe[4] to assess their oral, dental and periodontal status (Figure 10).

Figure 8. Examination Instruments.

This study was a randomized clinical study (RCS). It was conducted at Yeditepe University, Faculty of Dentistry, and Postgraduate Clinic of Periodontology during the time period (2016-2017).

This project was approved by the Yeditepe University School of Medicine and conducted in compliance with the ethical principles of the Health Sciences Research Ethics Board (REB) (Decision No.: 653/2016) (Appendix I). Before the enrollment, all subjects were given oral and written instructions; and informed about the used products, the purpose, reason, duration, possible benefits and possible adverse effects of study participation. Participation of subjects in this study was voluntary. All subjects willing to participate in the study received and signed an informed consent form prior to the study procedures (Appendix II).

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[1] No.5 dental mirror, Savanah®, Savanah, Georgia, USA
[2] No.23 dental explorer, Hu-Friedy Mfg. Co., Chicago, USA.
[3] Tweezer, Schwert®, A. Schweickhardt GmbH & Co. KG, Seitingen/ Oberflacht, Germany
[4] Periodontal probe, Hu-Friedy Mfg. Co., Chicago, USA.

3.1.1. Inclusion Criteria

Subjects eligible for this study were as follows:

1. Males and females aged ≥18 years.
2. Periodontally healthy subjects with at least 24 teeth (excluding third molars).
3. No predisposing oral factors causing any local irritation and plaque retention.
4. No presence of systemic diseases.
5. No pregnancy or breastfeeding.
6. No history of drug abuse.
7. No use of medications such as antiinflammatory drugs or antibiotics within previous 3 months.
8. No continuous use of oral antiseptics prior to the study.
9. Non-current smoker or previous smoker.

3.2. The Used Products in the Study:

The test product of aloe vera-containing mouth rinse was Lemon-Mint Power Rinse with Oxygen®[5] (ALV+ClO_2) (Figure 11). It contains 0.1% of stabilized ClO_2 in a form of sodium chlorite ($NaClO_2$) combined with zinc acetate as the main active ingredient in this formulation with additional active ingredients as; aloe vera leaf juice (*Aloe Barbadenesis*), xylitol, and a blend of essential oils of citrus and mint (lemon, orange, grapefruit, bergamot, lime, and spearmint) (Table 2). Other ingredients such as natural flavor, sucralose, PEG-40 hydrogenated castor oil, sodium citrate, citric acid and sodium hydroxide were added. It does not contain sugars, gluten or any irritating substance like alcohol, sodium lauryl sulfate SLR, paraben, or other chemicals like coloring agents or chemical preservatives (347).

The used positive control was 0.2 % CHX gluconate mouth rinse (Klorhex®[6]) was used as a positive control with additional ingredients such as; water, 20% glycerin, 0.2% lemon scent and 0.02% mint scent (Figure 11). In addition, distilled water[7] solution was used as a negative control (Table 3).

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[5] Lemon-Mint Power Rinse with Oxygen®, Oxyfresh Worldwide Inc., Spokane, Washington, USA.
[6] Klorhex ®, Drogsan Pharmaceuticals, Ankara, Turkey.
[7] Distilled water, (Onur Kimya Industry and External Trade. LTD. Company, Istanbul, Turkey.

Table 2. The Active Ingredients of the Tested Mouth Rinse (ALV+CO_2).

Active Ingredients	Action
Oxygene® (Sodium Chlorite; Stabilized ClO_2)	Antimicrobial action Disrupts bacterial biofilm colonization Oxidizes volatile sulfur compounds Eliminates oral malodour
Zinc	Anti-VSCs; counteracts the toxicity of volatile sulfur compounds and inhibits oral odor
Aloe Vera	Soothing relief for soreness and inflammation
Xylitol	Inhibits the growth of cariogenic bacteria
Essential Oils (Citrus and mint oils)	Natural flavors with a blend of soothing effect

Table 3. The Basic Information about the Used Mouth Rinses in the Study.

Mouth rinse	Active Ingredients	Code	Regimen
Test	**Chlorine Dioxide, Aloe vera, Zinc acetate, Xylitol, Citrus and Mint oils (ALV+ClO_2)**	**A**	**10 ml, 60 sec, 2×1**
Positive control	**0.2% Chlorhexidine gluconate (CHX)**	**B**	**10 ml, 60 sec, 2×1**
Negative Control	**Distilled water (DW)**	**C**	**10 ml, 60 sec, 2×1**

Figure 9. Used Mouth Rinses in the Study.

3.3. Sample Size Calculation

The sample size was verified according to PS® (Power and Sample Size Program) and based on the data from a previous study of a 4-day supragingival plaque regrowth model (37). Considering 80% power and α error of 0.05 with a standard deviation of 0.36 and mean difference of PI scores of 1.11 between the test and control group, a sample size of 30 subjects were needed. In order to compensate the drop out during the study period, a 10% of drop out was considered. Therefore, 33 subjects were included in the present study.

3.4. Study Groups

The allocation of mouth rinse products ALV+ClO_2, CHX, and DW was carried out by a second person who was not directly involved in the research project into codes of A, B, C, respectively. Treatment groups and treatment sequences were as the follows (Figure 12).

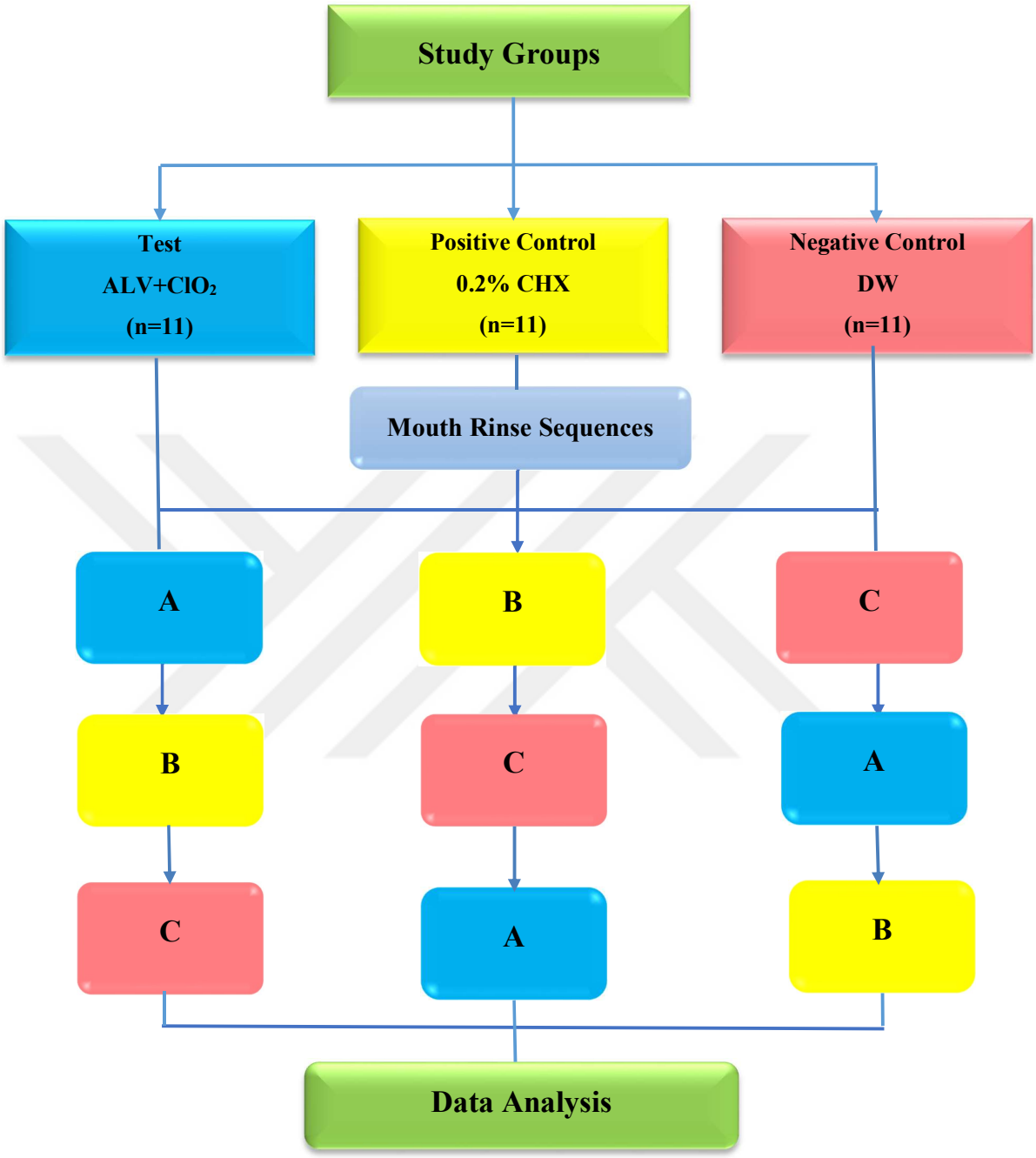

Figure 10. Flowchart of the Sequences of the Mouth Rinses in Each Group.

3.5. Study Design

The present study was a double-blind, randomized (3x3) Latin square controlled, crossover design in a 4-day non-brushing model. The supragingival plaque regrowth model that proposed by Addy *et al.* (124) was chosen to evaluate *de novo* plaque formation in a crossover design with three-sequence, three treatment periods and ten days of washout period to avoid the carry-over effect. The flow chart of this study is shown in Figure (14).

Thirty three participants were equally and randomly assigned into three treatment groups (11 participants in each group) according to a computer-based randomization table (www.randomizer.org / Copyright ©1997- 2011 by Geoffrey C. Urbaniak and Scott Plous). A printed schedule was given for every participant. For easier communication, every participant had been reminded with a text message to come on a schedule via electronically generated study group.

The present study was conducted as a double-blind trial in which neither the participants nor the investigator was aware of the treatments received in order to minimize the potential bias. Also, this level of blinding was maintained throughout the conduct of the study. The test and control mouth rinses were dispensed in identical opaque plastic bottles[8] of 200 ml that labeled with codes (A, B, C) (Figure 13).

Figure 11. Allocated Mouth Rinses Labeled with codes A, B, C.

[8] **Bottle, Drogsan Pharmaceuticals, Ankara, Turkey**

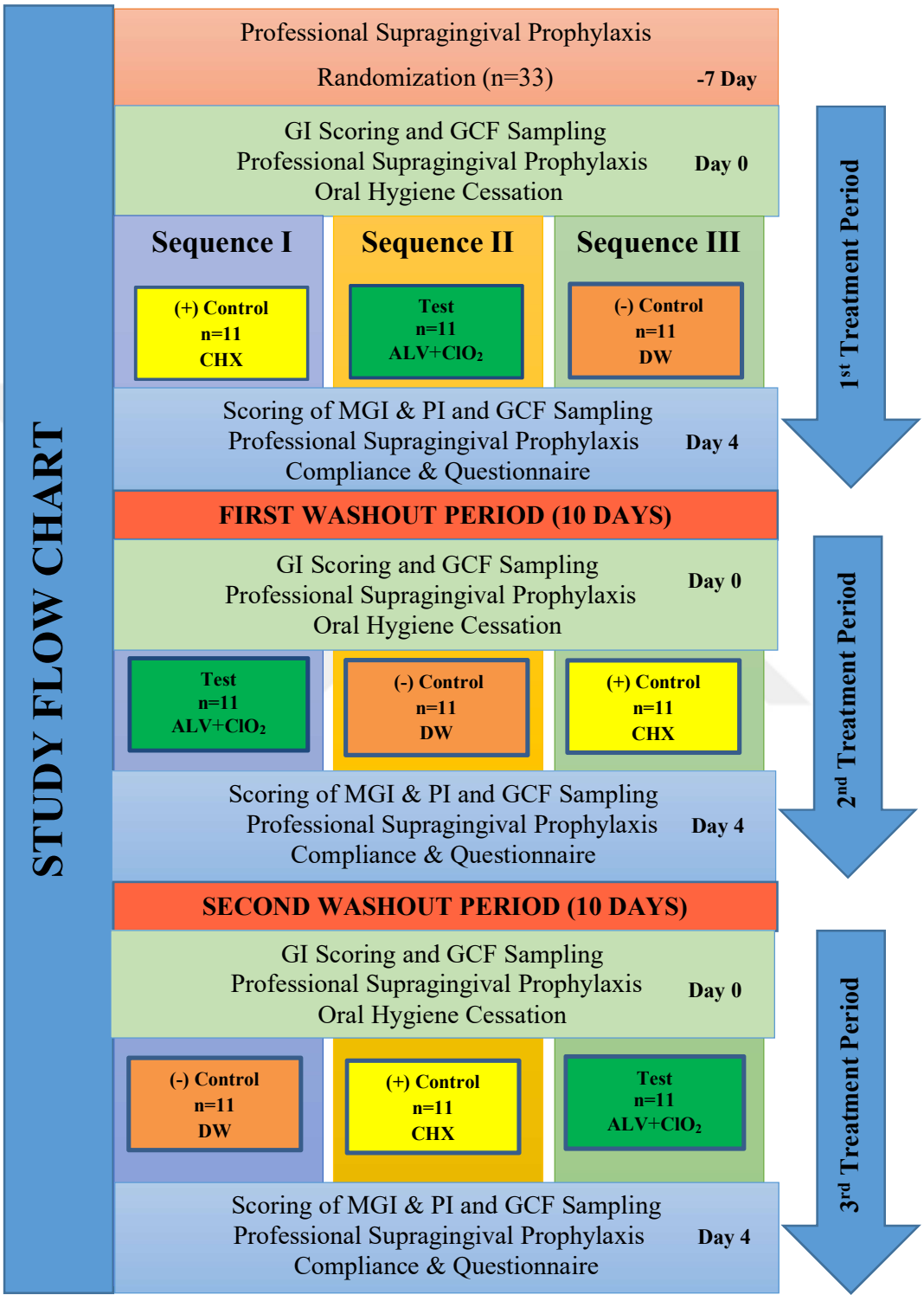

Figure 12. The Flowchart of the Study Design.

Every participant received a supragingival prophylaxis (SGP) and given the oral hygiene instructions one week prior to the commencement of the study in order to achieve healthy gingival status. Subjects were informed to perform only mechanical plaque control by a manual toothbrush[9] twice a day with an adjunctive toothpaste[10] and besides using dental floss[11] once a day as the oral hygiene measure before the commencement of the study (pre-experimental period) and during the washout periods (Figure 15).

Figure 13. Standard Oral Hygiene Products for Every Participant.

3.6. Clinical Indices and Scoring Criteria

Scoring of PI at day 4 (D4) was considered as the primary outcome variable. In addition, evaluation of gingival inflammation by using gingival index (GI) at baseline and day 4 (D0, D4) respectively and estimating the changes in the GCF volume were considered as a secondary variables since they may be affected by plaque accumulation and it also used to reassure the healthy gingival conditions at the beginning of every study period .

Measurements were performed on day 0 and day 4 of every treatment period and recorded by the same examiner into the data sheet (Clinical assessment form) (Appendix III).

[9] **TePe Supreme, TePe®, Oral Hygiene Products AB, Malmö, Sweden.**
[10] **Sensodyne® with fluoride, GlaxoSmithKline plc. Brentford, Middlesex, UK.**
[11] **Oral B Satin Floss, Oral-B®, Oral-B laboratories, County Kildare, Ireland.**

3.6.1. Gingival Index

The mean gingival index (GI) on day 4 of each experimental period was considered the secondary outcome variable. The clinical scoring procedure that used to assess gingival inflammation was modified gingival index by Lobene (MGI) (201). It is a noninvasive method of scoring that estimates gingival inflammation by visual assessment according to a numerical scale illustrated in Figure 16. Each tooth is examined visually and scored in 6 areas: 1) mesiofacial, 2) midfacial, 3) distofacial, 4) mesiolingual, 5) midlingual, 6) distolingual. The maximum score per tooth is 24 whereas the maximum index per tooth or subject is 4. All teeth were included except third molars and those teeth with prosthetic crowns or cervical restorations. Gingival index score for each subject is calculated by adding all the individual plaque scores and dividing this sum by the total number of measurements (the number of teeth scored multiplied by six). While the mean GI for each group was calculated by adding all individual means GI and divided by the number of subjects.

Criteria for Modified Gingival Index Scoring by Lobene

0 = **Normal or absence of inflammation**.

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

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

3 = **Moderate inflammation**, 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.

Figure 14. Criteria for Modified Gingival Index Scoring by Lobene (201).

3.6.2. Plaque Index

Mean plaque index (PI) on day 4 (D4) of each experimental period was considered the main primary outcome variable. The clinical scoring procedure aimed to assess supragingival plaque formation using a Turesky modification of the Quigley-Hein Plaque Scoring Index (QHI) (202, 203). The modified Quigley-Hein Plaque Scoring Index requires the usage of a disclosing agent and it scores supragingival plaque formation according to a numerical scale illustrated in Figure 17 and 18. After using of disclosing agents[12], participants were asked to rinse with water. Then each tooth is examined visually and scored in six areas: 1) mesiofacial, 2) midfacial, 3) distofacial, 4) mesiolingual, 5) midlingual, 6) distolingual. The maximum score per tooth is 30 whereas the maximum index per tooth or subject is 5. All teeth are included except third molars and those teeth with prosthetic crowns or cervical restorations.

The Mean Plaque Index for each subject was calculated by adding all the individual plaque scores (six per tooth) and dividing this sum by the total number of measurements (the number of teeth scored multiplied by six). While the mean plaque index for each group was calculated by adding all the individual mean plaque index and divided by the number of subject in the group.

Criteria for Plaque Index Scoring by Turesky *et al.*

0 = No plaque present.

1 = Separate flecks of plaque at the cervical margin.

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

3 = A band of plaque wider than 1 mm but covering less than one-third of the surface.

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

5 = Plaque covering more than two-thirds of the surface.

Figure 15. Plaque Scoring Criteria for Turesky Modification of Quigley-Hein Index (203).

[12] **TePe PlaqSearch™, TePe®, Oral Hygiene Products AB, Malmö, Sweden**

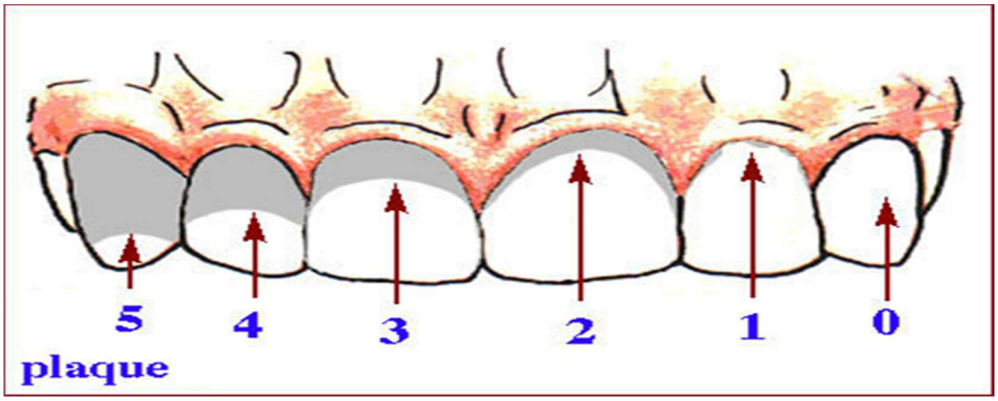

Figure 16. Illustration of the Scoring Criteria of Turesky Modification of Quigely-Hein Index (204).

3.7. Clinical Procedures

The clinical procedures of the present study can be divided into two periods: pre-experimental & experimental. Every participant had been involved in the study for a total period of 39 days.

At day -7, prior to the commencement of the study, all participants had received a supragingival prophylaxis (SGP) including supragingival scaling using cavitron[13], polishing using polishing paste[14] with polishing brush[15] which mounted on low-speed handpiece[16], interdental cleaning using an interdental floss, and cleaning of the distal surface of last molar using sterile gauze strips[17].

The purpose of this pre-experimental period was to standardize the oral care measures during the study period for all participants. They were instructed to brush their teeth with Modified Bass Technique regularly twice a day using a standard manual tooth brush with non-antimicrobial containing toothpaste. In addition, flossing once a day using dental floss.

Participants were also advised not to use any mouth rinse, spray, gel, lozenge, chewing gum or tablet containing antimicrobial agents for the whole pre-experimental and experimental period (during 39 days of study period).

Experimental period consisted of three different 4-day treatment periods (tx) together with two washout periods of 10 days.

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[13] Cavitron, Woodpecker® manufacturer, Guilin, Guangxi, China.
[14] Prophylaxis Paste, Detartrine®, Stepodont, Saint-Maur-des-Fossés Cedex, France.
[15] Polishing brush, Savannah®, Georgia, USA.
[16] Intramatic Motor 181 DBN Air Micromotor, KaVo do Brasil Ind.Com. Ltda, Santa Catarina, Brazil.
[17] Sterile guaze strips, Mediteks Incorporated, Istanbul, Turkey.

On day 0 of each treatment period, GI was recorded on the data sheet and then GCF samples were collected from each participant and the GCF volume was estimated by using periotron 8000® device[18]. Subsequently, PI was evaluated using a disclosing agent as mentioned before, and finally, participants were received a supragingival prophylaxis to remove all supragingival plaque, calculus, and stains. They were then instructed to cease their usual oral hygiene procedures for the following 4 days and emphasized to only use of the allocated mouth rinse that was dispensed to them. Participants were asked to rinse with 10 ml of the allocated mouth rinse twice daily for 60 seconds without subsequent rinsing with water and no eating or drinking for a period of 30 minutes. All instructions were given in detail verbally as well as in writing instruction form (Appendix V).

On day 4, all participants were evaluated for oral and soft tissue status, followed by GCF sampling, GI and PI measurement along with intraoral photographs and then they received a supragingival prophylaxis. Any adverse effect that may happen during the treatment period was recorded and their attitude for the used products was evaluated by satisfaction questionnaire. Finally, the compliance of participants during the treatment period was estimated from the collected mouth rinse bottles. All the returned bottles were weighed by using Precision Scale[19]. The chronology of the experimental week is explained in Table 4.

3.7.1. Gingival Crevicular Fluid Sampling

Changes in GCF volume after each treatment period were considered as a secondary outcome variable that evaluates the gingival inflammation. Four single rooted-teeth (one tooth per each quadrant) were selected for GCF sampling. Samples were taken intercrevically at the interproximal surface of the same teeth at baseline and on day 4. GCF samples were collected using periopaper® strips[20]. The selected sample sites were gently dried with cotton and all supragingival plaque were removed carefully with a periodontal probe. The areas were carefully isolated using cotton rolls to prevent the contamination by saliva. The paper strips were inserted into the gingival crevice until mild resistance was felt or into a depth not more than 1mm and left for 30 seconds (Figure 20). Care was taken to avoid mechanical injury to the gingival tissues. Any strip contaminated by blood or exudate was discarded.

[18] Periotron 8000 Smithtown, New York, USA.
[19] Posch® P 115, PHYWE, Göttingen, Germany.
[20] Periopaper® Oraflow Inc., New York, USA.

Immediately after collection, the strips were positioned on a previously calibrated device then the values provided by the device were converted to actual volumes, expressed in µL, by referring to the correspondent calibration logarithmic curve.

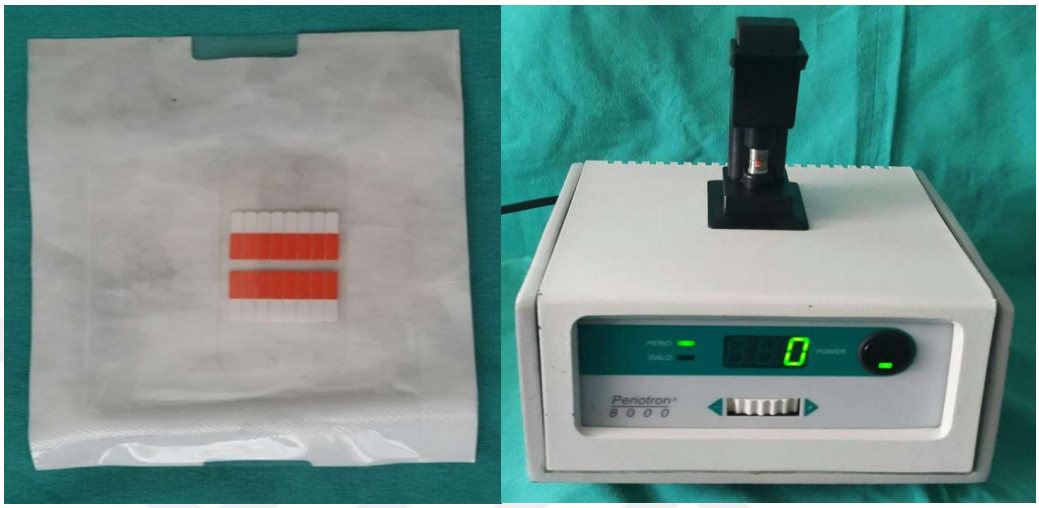

Figure 19. Periopaper® Strips and Periotron® 8000 Device.

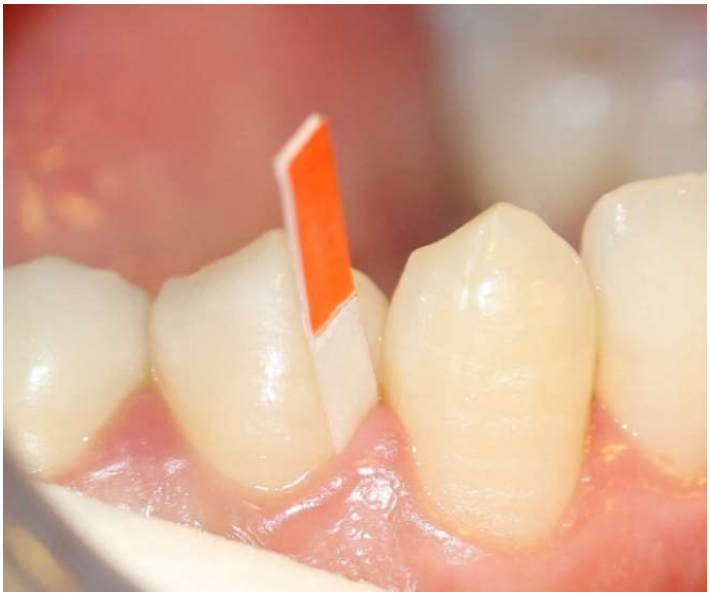

Figure 20. GCF Sampling Procedure.

Table 4. The Chronology of the Experimental Week.

Day	Morning	Night
Thursday Day 0	➢ **GI** ➢ **GCF sampling** ➢ **PI** ➢ **SGP and Oral hygiene cessation** ➢ **Allocation of Mouth rinses**	**1st Use**
Friday Day 1	**2nd Use**	**3rd Use**
Saturday Day 2	**4th Use**	**5th Use**
Sunday Day 3	**6th Use**	**7th Use**
Monday Day 4	**8th Use** ➢ **Oral and soft tissues status** ➢ **GI** ➢ **GCF sampling** ➢ **PI** ➢ **Intraoral photographs** ➢ **SGP** ➢ **Satisfaction Questionnaire** ➢ **Adverse effects** ➢ **Compliance**	**Starting the Washout period of 10 days**

3.8. Satisfaction Questionnaire

For every participant, the subject experience with the three different mouth rinses was evaluated at the end of every treatment period (Day 4) using a questionnaire (Appendix VI) and it is summarized in Table (5). The questionnaire was performed to evaluate their attitudes with regard to the product used using a visual analogue scale (VAS) (305). The questions were evaluating the taste perception, duration of the taste, alteration of taste, sensitivity, burning sensation, dry mouth, numbness, staining and degree of 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.

Table 5. Complete Questions of the Satisfaction Questionnaire with VAS Score.

Paraphrase	Complete Question	With extremes	
		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 mouth rinse?	Not at all	Very much
Burning sensation	Did you experience a burning sensation in the mouth because of the mouth rinse?	Not at all	Very much
Dry mouth	Did you experience a dry mouth because of the mouth rinse?	Not at all	Very much
Numbness Feeling	Did you experience a numbness feeling in the mouth because of the mouth rinse?	Not at all	Very much
Staining	Did you experience staining on the teeth because of the mouth rinse?	Not at all	Very much
Cleanliness	Did you have the feeling that your teeth were clean for the last 4 days?	Not at all	Very much

3.9. Statistical Analysis

At the end of the experimental period, statistical analysis was performed by IBM SPSS® Statistics 22[21] software. 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 comparisons of the treatments 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 $p<0.05$.

[21] **SPSS®, IBM Corporation, New York, USA.**

4. RESULT

Thirty-three participants who met the inclusion criteria and provided consent to participate were enrolled in this randomized clinical trial. All of the participants completed the study period. No adverse-effects were reported or observed throughout the study. Intra-oral pictures of representative cases are shown in Figures (21-26).

4.1. Demographic Characteristics of Subjects in the Study

33 participants (15 males, 18 females) aged between 21-26 years were included. Demographic characteristics of the subjects are shown in Table 6.

Table 6. Demographic Characteristics of the Subjects.

Number of subjects		**33**
Gender (M/F)		**15M/18F (45.5% M / 54.5% F)**
Age	**Mean Age**	**22.94**
	Age range	**21-26 yrs.**
	Mean Age±SD	**22.30±1.89 yrs.**
PI		**1.84±0.45**
GI		**1.43±0.26**
Number of PD>3mm		**—**

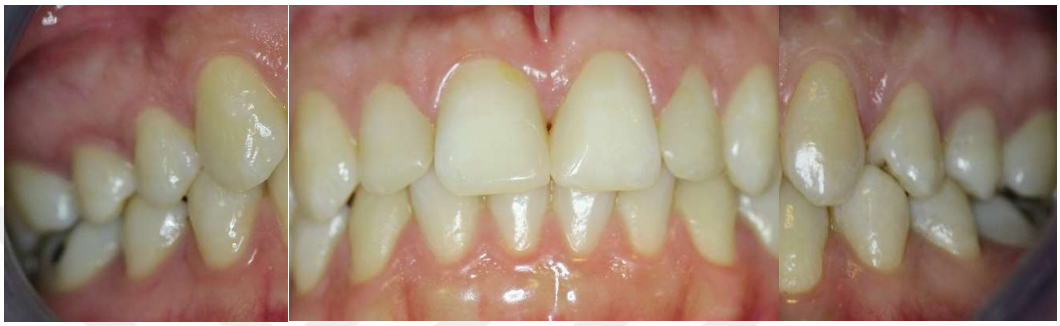

Figure 17. The Clinical View of ALV+ClO_2 Treatment on Day 0.

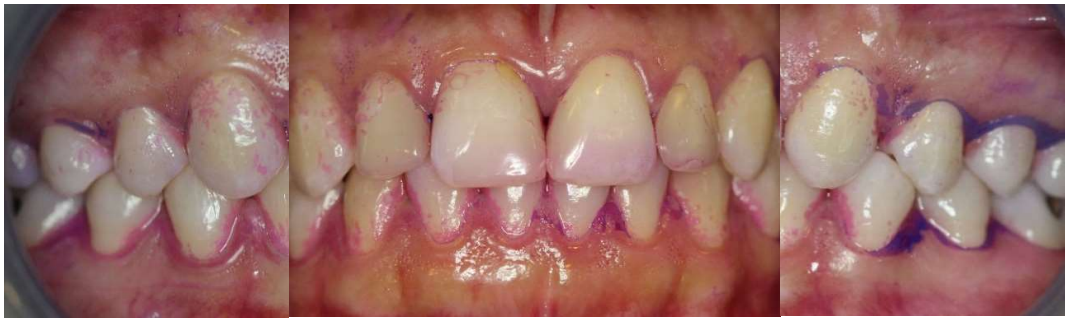

Figure 18. The Clinical View of ALV+ClO_2 Treatment on Day 4.

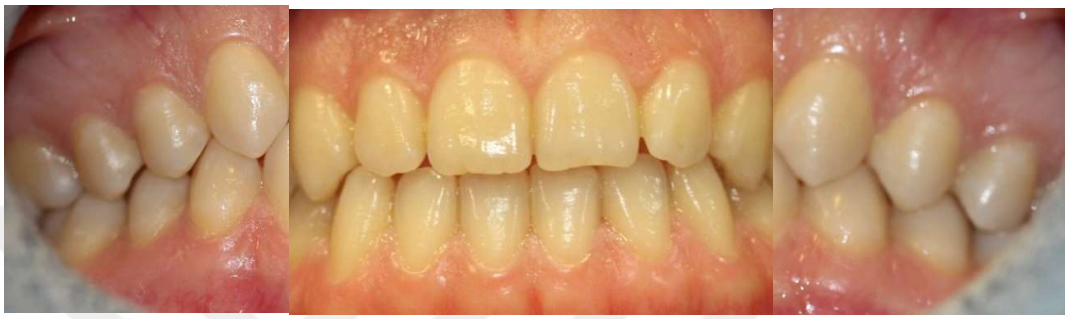

Figure 19. The Clinical View of CHX Treatment on Day 0.

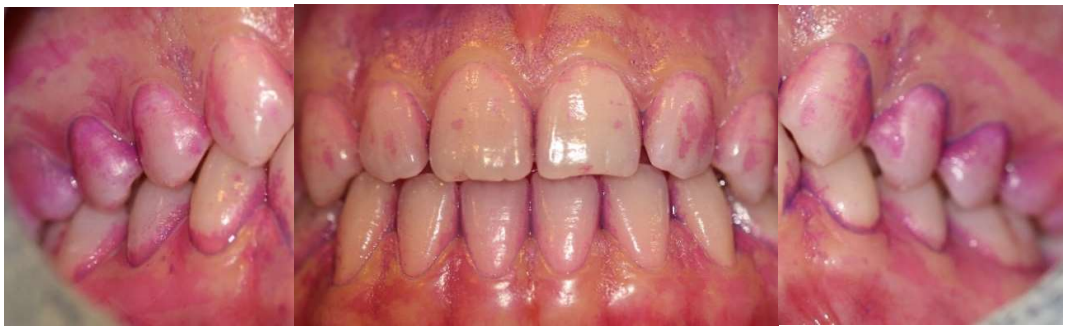

Figure 20. The Clinical View of CHX Treatment on Day 4.

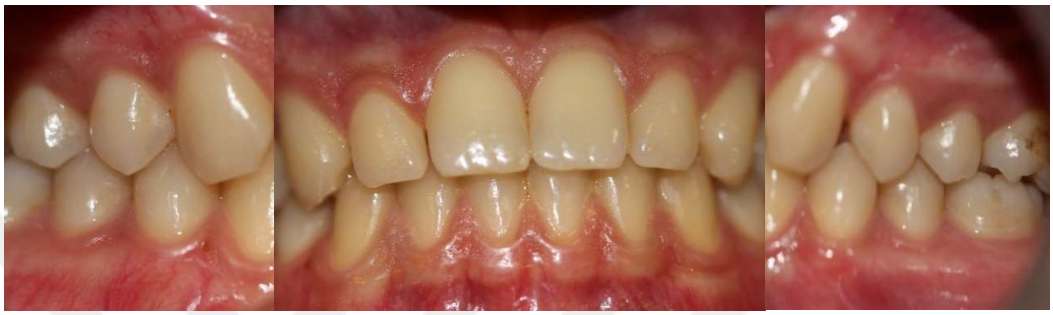

Figure 21. The Clinical View of DW Treatment on Day 0.

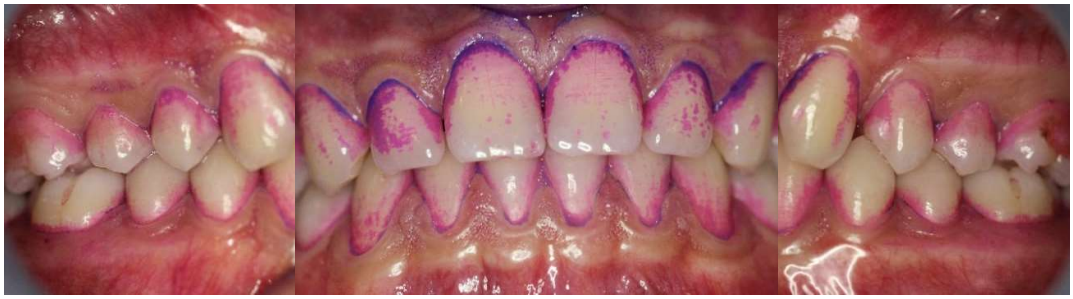

Figure 22. The Clinical View of DW Treatment on Day 4.

4.2. Clinical Results

The mean values and standard deviations (SD) of PI, GI and GCF parameters are presented in Tables 7, 9 and 10, respectively.

4.2.1. Plaque Index

On day 4, the mean PI values were detected 1.92±0.38, 1.58±0.47 and 3.05±0.65 in ALV+ClO_2, CHX, and DW treatments, respectively (Table 7).

Inter-treatment multiple comparisons of the mean PI values demonstrated a statistically significant difference between the treatments ($p=0.000$, $p<0.05$) (Table 7). Subsequent double comparison of the mean PI values revealed statistically significant differences between (ALV+ClO_2)-CHX, (ALV+ClO_2)-DW, and CHX-DW treatments in favor of the positive control ($p=0.01$, $p=0.000$, $p=0.000$, $p<0.05$), respectively (Table 8).

Table 7. Mean Values and Standard Deviations of the PI on Day 4.

PI	ALV+ClO_2	CHX	DW	P[1]
	Mean±SD	Mean±SD	Mean±SD	
Day 4	1.92±0.38	1.58±0.47	3.05±0.65	0.000*

[1]*Repeated measures of variance, *p<0.05.*

Table 8. Inter-treatment Double Comparisons of PI Values.

PI	(ALV+ClO_2)-CHX	(ALV+ClO_2)-DW	CHX-DW
	p	p	p
Day 4	0.001*	0.000*	0.000*

*Bonferroni test, * p<0.05*

4.2.2. Gingival Index

In ALV+ClO_2 treatment, mean GI values were detected 0.45±0.23 on day 0 and 0.46±0.21 on day 4. Mean GI values in CHX treatment were detected 0.36±0.26 and 0.41±0.23 whereas in DW treatment they were detected 0.46±0.33 and 0.54±0.30 on day 0 and day 4, respectively (Table 9).

In ALV+ClO_2, CHX and DW treatments, no statistically significant changes were detected between day 0 and day 4 (p=0.646, p=0.314, p=0.164; p>0.05) (Table 9).

On day 0 and on day 4, inter-treatment multiple comparisons and changes of the mean GI values from day 0 to day 4 revealed no statistically significant differences between the treatments (p=0.179, p=0.128, p=0.624; p>0.05) (Table 9).

Table 9. Mean Values and Standard Deviations of the GI Values on Days 0 and 4.

GI	ALV+ClO_2	CHX	DW	[1]p
	Mean±SD	Mean±SD	Mean±SD	
Day 0	0.45±0.23	0.36±0.26	0.46±0.33	0.179
Day 4	0.46±0.21	0.41±0.23	0.54±0.30	0.128
Difference	0.02±0.20	0.05±0.26	0.08±0.33	0.624
[2]p	0.646	0.314	0.164	

*[1]Repeated measures of variance, [2]Paired samples t-test, *p<0.05.*

4.2.3. GCF Volume

In ALV+ClO_2 treatment, mean GCF volume was detected 0.52±0.18 on day 0 and 0.63±0.17 on day 4. Mean GCF volume in CHX treatment was detected 0.56±0.11 and 0.57±0.21 whereas in DW treatment it was detected 0.52±0.19 and 0.74±0.25 on day 0 and day 4, respectively (Table 10).

In ALV+ClO_2 and DW treatments, increase of GCF volume was statistically significant between day 0 and day 4 ($p=0.000$, $p=0.000$; $p<0.05$). In CHX treatment, no significant difference was detected between day 0 and day 4 ($p=0.865$; $p>0.05$) (Table 10).

Inter-treatment multiple comparison of GCF volume on day 4 and changes of the mean GCF volume from day 0 to day 4 revealed statistically significant differences between the treatments ($p=0.027$, $p=0.011$; $p<0.05$) (Table 10). Subsequent double comparison of GCF volume revealed significant difference between CHX-DW treatments ($p=0.021$; $p=0.008$; $p<0.05$) (Table 11). However, no significant differences were observed in (ALV+ClO2)-CHX and (ALV+ClO2)-DW treatments ($p=0.153$, $p=0.126$; $p>0.05$) (Table 11).

Table 10. Mean Values and Standard Deviations of the GCF Volume on Days 0 and 4.

GCF	ALV+ClO_2	CHX	DW	P[1]
	Mean±SD	Mean±SD	Mean±SD	
Day 0	0.52±0.18	0.56±0.11	0.52±0,19	0.181
Day 4	0.63±0.17	0.57±0.21	0.74±0.25	**0.027***
Difference	0.10±0.13	0.01±0.24	0.23±0.29	**0.011***
P[2]	**0.000***	0.865	**0.000***	

*[1]Repeated measures of variance, [2]Paired samples t-test,*p<0.05*

Table 11. Inter-treatment Double Comparisons of the GCF Values.

GCF	(ALV+ClO_2)-CHX	(ALV+ClO_2)-DW	CHX-DW
	p	p	p
Day 0	1.000	1.000	0.410
Day 4	0.560	0.109	**0.021***
Difference	0.153	0.126	**0.008***

*Bonferroni test, * p<0.05*

4.3. Satisfaction Questionnaire

The mean values for taste perception of ALV+ClO_2, CHX, and DW treatments were 6.82±1.31, 3.33±2.46 and 5.15±2.35, respectively. The taste duration of ALV+ClO_2, CHX, and DW were scored 4.55±1.86, 6.67±1.91 and 0.12±0.42, respectively. Altered taste sensation scored 0.61±1.34, 5.27±3.2 and 0.09±0.38 in ALV+ClO_2, CHX and DW, respectively. Sensitivity was scored 0.82±2.05, 1.79±2.65 and 0.09±0.38 in ALV+ClO_2, CHX, and DW treatments, respectively. Burning sensation was perceived with ALV+ClO_2 and CHX as 0.82±2.05 and 3.82±3.51, respectively. Mouth dryness was scored 0.67±1.29, 3.06±3.53 and 0.09±0.38 in ALV+ClO_2, CHX, and DW treatments, respectively. Numbness was sensed only in ALV+ClO_2 and CHX treatments as 0.55±1 and 4.18±3.19, respectively. Staining was perceived only in CHX and DW as 1.33±2.62 and 0.09±0.38, respectively. Mouth cleanliness was sensed with ALV+ClO_2 and CHX treatments as 3.48±1.99 and 5.55±3.15, respectively (Table 12).

Multiple comparisons of the evaluated parameters in SQ revealed significant differences between the treatments ($p=0.000$, $p=0.000$, $p=0.000$, $p=0.002$, $p=0.000$, $p=0.000$, $p=0.000$, $p=0.002$, $p=0.000$, $p<0.05$) (Table 12).

Regarding the following parameters: the taste, taste duration, altered taste sensation, burning, mouth dryness and numbness, subsequent double comparison of the treatments revealed statistically significant differences between ALV+ClO_2-CHX treatments in favor of the test group ($p=0.000$, $p=0.000$, $p=0.000$, $p=0.000$, $p=0.000$, $p=0.000$, $p<0.05$) (Table 13).

For sensitivity parameter, double comparison of the treatments revealed a statistically significant difference between CHX-DW ($p=0.002$, $p<0.05$) (Table 13). Sensitivity was detected significantly higher in CHX treatment than DW (Table 13).

Regarding the staining parameter, double comparison of the treatments revealed statistically significant differences between (ALV+ClO_2)-CHX and CHX-DW treatments ($p=0.007$, $p=0.010$, $p<0.05$); however no significant difference was detected between (ALV+ClO_2)-DW ($p=0.180$, $p>0.05$) (Table 13).

For mouth cleanliness, double comparison of the treatments demonstrated statistically significant differences between (ALV+ClO_2)-CHX and CHX-DW treatments

in favor of the positive control (p=0.005, p=0.000, p<0.05). ALV+ClO_2 combination revealed higher cleaning ability than DW treatment (p=0.000, p<0.05) (Table 13).

Table 12. Evaluation of the Adverse Effects of the Treatments according to VAS Scores.

Questionnaire Results	(ALV+ClO_2)	(CHX)	(DW)	p
	Median±SD	Median±SD	Median±SD	
1. Taste	6.82±1.31 (7)	3.33±2.46 (3)	5.15±2.35 (5)	0.000*
2. Taste duration	4.55±1.86 (5)	6.67±1.91 (7)	0.12±0.42 (0)	0.000*
3. Altered taste sensation Food/Drink	0.61±1.34 (0)	5.27±3.2 (4)	0.09±0.38 (0)	0.000*
4. Sensitivity	0.82±2.05 (0)	1.79±2.65 (0)	0.09±0.38 (0)	0.002*
5. Burning	0.82±2.05 (0)	3.82±3.51 (3)	0±0 (0)	0.000*
6. Mouth Dryness	0.67±1.29 (0)	3.06±3.53 (1)	0.09±0.38 (0)	0.000*
7. Numbness	0.55±1 (0)	4.18±3.19 (5)	0±0 (0)	0.000*
8. Staining	0±0 (0)	1.33±2.62 (0)	0.09±0.38 (0)	0.002*
9. Mouth Cleanliness	3.48±1.99 (4)	5.55±3.15 (7)	0±0 (0)	0.000*

[1]*Friedman Test, *p<0.05*

Table 13. Inter-treatment Double Comparisons of the VAS Scores.

Questionnaire Results	(ALV+ClO_2)-CHX	(ALV+ClO_2)-DW	CHX-DW
Group Question	p	p	p
Taste	0.000*	0.001*	0.003*
Taste duration	0.000*	0.000*	0.000*
Altered taste sensation Food/Drink	0.000*	0.048*	0.000*
Sensitivity	0.113	0.063	0.002*
Burning	0.000*	0.042*	0.000*
Mouth Dryness	0.000*	0.024*	0.000*
Numbness	0.000*	0.007*	0.000*
Staining	0.007*	0.180	0.010*
Mouth Cleanliness	0.005*	0.000*	0.000*

*Wilcoxon sign test, * p<0.05*

5. DISCUSSION AND CONCLUSION

Dental plaque is considered the main etiological agent in the initiation of gingivitis and progression to periodontitis (1). In order to prevent the development of periodontal diseases, the colonization, proliferation and sequential layering of biofilm in order must be disrupted (206). Since gingivitis and periodontitis are a continuum of the same inflammatory disease, the prevention of gingival inflammation by plaque biofilm disruption prevents periodontitis (66). The prevention and treatment of gingivitis must be directed at the regular removal and disruption of this continually forming biofilm, which is challenging (207).

Mechanical plaque removal is widely regarded as a highly effective mean for controlling the progression of dental caries and periodontal diseases (8, 107, 113). However, for many reasons, this mechanical routine does not appear enough for the vast majority of patients as supported by incidence and prevalence data (208).

Chemotherapeutic agents can be used as adjunct to mechanical plaque control to provide an additional benefit in helping in plaque and gingivitis control (209, 210). The daily use of an effective antiplaque/antigingivitis mouth rinse is well supported by a scientific rationale and can be a valuable adjunctive component of oral hygiene regimens as well as a method for delivering antiplaque agents to mucosal sites throughout the mouth that harbor pathogenic bacteria capable of recolonizing supragingival and subgingival tooth surfaces (211).

Chlorhexidine is considered as a gold standard in the chemical control of dental biofilm as it has the most superior antiplaque activity. This antiplaque activity can be attributed to its substantivity or its persistence at dental and oral surfaces; around 12 hours. The substantivity of CHX prolongs its dual and immediate antibacterial effect; bacteriostatic and bactericidal (15, 147). CHX-containing mouth rinses have demonstrated significant reductions in plaque and gingivitis in short and long-term clinical studies (13). However, CHX has an unpleasant flavor and it causes pigmentation of teeth and restorations, taste alterations, increased supragingival calculus formation, and it may be associated with mucosal desquamation. Therefore, long-term daily use of CHX is not recommended and alternative substances that present efficacy in biofilm control with reduced adverse effects is considered as an important therapeutic approach.

Natural oral products have made of natural, animal or plant-based ingredients that emphasize holistic health and wellness. They do not contain alcohol, sugar, artificial colors and sweeteners (such as saccharine), stannous fluoride, CPC, SLS, and harsh chemical preservatives and dyes (141). Also, natural compounds can act in a synergistic manner within the human body and can provide unique therapeutic properties with minimum or no adverse effects (212). In the present study, tested mouth rinse is one of the commercially available natural oral products.

Recently, there is great enthusiasm for synergistic therapy. The synergistic combination of two or more agents can overcome toxicity and other adverse effects associated with high doses of a single agent. Also, it allows using low doses of the therapeutic agents and provide a multi-target mechanism (50). Therefore, in this study, an attempt has been made to evaluate the clinical effectiveness of a natural mouth rinse containing a combination of ALV, ClO_2, Zn, XYL, and CMO in terms of plaque inhibition and subject perception when used as the only oral hygiene measure.

In the current study, a prospective double blind randomized controlled clinical trial (RCT) design was adopted since it provides a higher level of evidence for chemotherapeutic agents used for chemical plaque control (213). The four-day plaque regrowth model was chosen for the present trial as it is the first screening method and typical evaluation of new oral hygiene formulations and products (214). Plaque regrowth studies may have limitations in terms of providing long-term results and insufficient to evaluate the antigingivitis efficacy. However, it is adequate for studying the antiplaque efficacy and early occurrence of adverse effects (140). It has been employed in numerous investigations for assessing the plaque-inhibitory activity of formulation *per se* and determining the relative efficacy of different formulations (28, 36, 37, 39, 124, 125, 131). Since the measurable volume of plaque accumulation is reached after 4-5 days of oral hygiene cessation, a four-day was chosen as the length of experimental period (5). In this study model, *de novo* plaque formation can be evaluated in a short period without causing detectable harm to the study subjects.

As other short-term studies, the four-day regrowth model study which involves suspension of normal oral hygiene, provides important information on the chemical plaque-inhibitory properties of agents without introducing tooth brushing as an additional source of variation (214). Other advantages offered by the adaptation of this model

involves the removal of confounding variables such as the Hawthorne effect; improved oral hygiene consequent upon involvement in the clinical trial, the influence of the prestudy prophylaxis and the possible interactions between the mouth rinse and toothpaste ingredients. In addition, this study design measures the plaque regrowth under the influence of test solution from a zero plaque score at baseline and avoids confounding influence of tooth brushing, which is highly variable between individuals. Therefore, with no oral hygiene, plaque regrowth models provide information about the maximum plaque inhibitory effect that might be obtained with an agent (215, 216). Consequently, if no plaque inhibition observed, no further effect of rinsing can be expected in other study models where oral hygiene is performed (125).

The present study was designed as crossover instead of parallel one as it is more standardized and recommended method for a four-day plaque regrowth study (119). A particular advantage of a crossover design is that each subject acts as his/ her own control. Accordingly, it reduces the influence of confounding factors that arise because of individual variables which affect the plaque formation like salivary flow and composition, existing plaque retention sites, pre-existing gingivitis, dietary habits, and the composition of pellicle (217, 218). Also, optimal crossover designs require fewer subjects than non-crossover designs without losing their power. The main disadvantage of the crossover design over a parallel one is the possibility of a carry-over effect; the effect of one treatment on the outcome lasting in the following period, which can invalidate the results (219). It was reported that a 10-day of washout period is preferable to eliminate the residual effect of CHX (220). Therefore, to minimize the "carry-over effect" between the treatment periods, a washout period of 10-day was chosen in our study. Study participants were chosen from dental faculty students as they are well educated, cohesive, and more sensitized to oral hygiene protocols and would understand and comply with a better way to treatment regimens (28).

In this study, the antiplaque action was assessed with the clinical indices using quantitative parameters; PI as the primary outcome variable; GI, and GCF volume as the secondary variables as they are related to gingival inflammation which may be affected by plaque accumulation. Turesky modification of the Quigley and Hein index (202, 203) was used to assess plaque accumulation on the gingival third of the tooth. It is recognized as a reliable index for measuring plaque using an estimate of the area of the tooth covered by plaque. It provides a comprehensive method for evaluating antiplaque procedures such

as tooth brushing and flossing as well as chemical antiplaque agents (221). Gingival inflammation was evaluated on the marginal and papillary gingival units on scorable teeth by using modified gingival index by Lobene (MGI); which introduced changes in the Löe and Silness GI through a non-invasive approach (no probing for bleeding assessment) and resetting the rating for mild and moderate inflammation (201). Also, the gingival inflammation further assessed by the quantitive evaluation of GCF volume through using a calibrated electronic device, since the GCF volume increases during gingivitis (127, 128).

Subjects were instructed to rinse their mouth twice daily with 10 ml of solution for a period of 60 seconds every 12 hours, after breakfast and dinner, and they were asked not to rinse their mouth with water and not to eat or drink anything for half an hour. Similar amount and duration of mouth rinse administration were followed in many studies (15, 16, 39). Compliance to the study protocol was checked by asking the participants to return the bottles at the end of every four-day period.

To the best of our knowledge, the present study is the first plaque regrowth study evaluating the clinical efficacy of a combination natural mouth rinse containing; ALV, ClO_2, Zn, XYL, and CMO, on plaque and gingival inflammation reduction. The results showed that both test and positive control mouth rinses were efficient in plaque reduction. However, plaque reduction was significantly higher in CHX mouth rinse compared to ALV+ClO_2 combination.

Although ALV+ClO_2 mouth rinse score was seen less than CHX for mouth cleanliness, it scored obviously better in the satisfaction questionnaire, especially for taste perception and tooth staining. ALV+ClO_2 mouth rinse had a less negative impact on taste sensation, mouth dryness, sensitivity, burning, and numbness when compared to 0.2% CHX solution. No adverse events were observed on clinical examination or reported by the patients. Participants demonstrated a higher subjective preference for the tested mouth rinse, also, had a better compliance with the regular use of ALV+ClO_2 proving its acceptability.

In the English literature, no plaque regrowth study with a similar study design has been conducted to investigate the clinical efficacy of the ALV+ClO_2 rinse. Review of the literature reveals a few "home-use studies" in subjects with chronic periodontitis (CP).

After oral prophylaxis, these studies evaluated the tested mouth rinse as an adjunct to tooth brushing for 2, 4, and 12-month periods (222-224).

Splinder and Splinder (222) reported that the usage of stabilized ClO_2 toothpaste and mouth rinse combination twice daily for a period of 60 days yielded a greater efficacy in improving PI, GI, and BOP scores compared to phenolic-based rinse. However no significant difference was detected between the groups in terms of probing depth (PD) reduction.

Mani *et. al.* (223) assessed and compared the clinical and antimicrobial effects of ALV+ClO_2 toothpaste and mouth rinse combination regimen along with a nutritional dietary supplement of the Coenzyme Q10 (CoQ10) complex in 4-month home-use study to conventional toothpaste and mouth rinse containing essential oils. The highest decrease in GI, PI, CAL and PD was observed in ALV+ClO_2 +CoQ10 complex regimen together with the highest decrease of *A. actinomycetemcomitans*, *Fusobacterium nucleatum*, *P. gingivalis* and *P. intermedia*. The significant periodontal benefit from the combination of ALV+ClO_2 and CoQ10 supplement was attributed to the oxygenation of the anaerobic environment that lead to lowering all key periodontal pathogens and disruption of the biofilm.

With similar study design, Saini (224) reported significant reductions in clinical and microbiological parameters in CP patients from baseline to 12 months by using ALV+ClO_2 toothpaste and mouth rinse combination. The subjects under test group showed significantly higher reductions in PI, GI, and CFU of *A. actinomycetemcomitans, F. nucleatum*, *P. gingivalis* and *P. intermedia* except CAL and PD compared to control group (conventional alcohol-based toothpaste and mouth rinse).

Although the results of the previous studies are promising, these results cannot be directly compared to that of the present study due to different study designs and durations, study models, study populations as well as the rinsing regimen. These heterogeneities limit the comparisons of the study results. In the present study 0.2% CHX gluconate was used, the gold standard for the chemical plaque control, as a positive control. However, CHX mouth rinse cannot be used in the long-term studies (126). Therefore, previous home-use studies used essential oil mouth rinses as a control, which has less antiplaque and antigingivitis efficacy compared to CHX (222, 223).

Previous short-term studies reported that ClO_2 mouth rinse alone significantly reduced plaque accumulation (36, 181, 197). However, when compared to CHX, CHX showed better PI and GI reduction than ALV+ClO_2 combination. The findings of the present study are in line with a 3-day plaque regrowth study of Paraskevas et al. (15). In contrast, in 3 short-term studies, the ClO_2 containing mouth rinse exhibited similar effect to CHX rinse on PI and GI reduction (16, 37, 39). However, in one of these studies, 0.12%CHX solution was used whereas 0.2%CHX was used in the other study (37, 39). The concentration of CHX was not mentioned in the third study of experimental gingivitis model which makes the comparison of the results impossible (16). In a 5-day plaque regrowth study by Gultschin (36), a placebo solution has been used as the only control. Two different concentrations of metastabilized chlorous acid/ClO_2 resulted in significant plaque reduction without a significant change in the salivary bacterial count, compared to the placebo group.

In accordance with the findings of the studies using ClO_2 rinse, four randomized control trials demonstrated that a range of 98-100% of ALV mouth rinse has a similar effect to 0.12% and 0.2%CHX on PI and GI reduction (22, 24, 25, 27). However, one of these clinical trials conducted by Karim *et al.* (24) has a different study design since the daily tooth brushing was not refrained. In another study, statistically significant difference was detected between ALV and 0.2%CHX mouth rinses in PI reduction, but no significant difference was detected in GI scores (22). Conversely, ALV resulted significantly less plaque and gingivitis reduction than 0.2%CHX in other short-term studies (23, 26, 28).

A randomized, clinical trial with a parallel design by Yeturu *et al*. (16) demonstrated a highly significant reduction of PI and GI scores in subjects with fixed orthodontics over 15 days periods using CHX, ClO_2, and ALV mouth rinses. CHX group demonstrated significantly higher reductions in PI and GI scores when compared to the ALV group. However, no significant difference was observed between CHX and ClO_2 with respect to mean reduction of PI and GI scores. The highest percentage of plaque reduction was detected in CHX group (31.59%) followed by ClO_2 group (30.29%) and ALV group (20.38%). Similarly, the percentage reduction of gingival scores was maximum in CHX group (16.30%) followed by ClO_2 group (12.22%) and ALV group, which showed the least reduction (9.88%). However, the results of the study may be limited by the small sample size. In addition, the concentrations and sources of the mouth rinses were not given in detail.

Plaque reduction achieved by ALV+ClO_2 can be attributed to synergistic effect from combining many agents with plaque inhibitory effect and different target mechanisms. However, literature is lack off data analyzing the possible synergistic or additive effect of this combination. In addition, the manufacturer does not provide any information related to the concentration of the active agents in the tested formulation, which is very important since the effect of any therapeutic agent is dose-dependent. Sims and Zimmerman (51) investigated the bactericidal and fungicidal effects of various percentages of ALV gels *in vitro* and found that the dramatic effects of the aloe gel were not apparent until there was at least a 70% concentration of the gel. This can explain the conflicting results from *in vitro* and *in vivo* studies using different concentrations of ALV. Four studies compared the efficacy of herbal mouth rinse containing 20%ALV gel to CHX and EO mouth rinse (29, 30, 53, 54). Two of these studies were *in vivo* studies and revealed that CHX was significantly better than 20% ALV and EO in plaque and gingival inflammation reduction (29, 30). However, other two *in vitro* studies reported conflicting results. One study reported ALV gel to be significantly better than CHX and EO rinses (53). On the other hand, the second study found CHX was better than ALV gel and ALV gel to be better than EO rinse (54). Villalobos *et al.* (21) observed significant reductions in plaque and gingivitis scores after 30 days usage of mouth rinse containing 50% ALV combined with tooth brushing compared to placebo. Despite the additive effect of brushing, this finding was a result of using only ALV gel as the active ingredient.

The antiplaque action of the tested mouth rinse may result from the combined effect of the key ingredients; ClO_2, Zn, ALV, and XYL (200). However, no data available to explain the exact mechanism of antiplaque action and whether this combination result from synergistic or additive effect. As a summary of the previous studies, manufacturer's investigation and report data, it is stated that, ClO_2 breaks the double bond in sulfur-containing substrates, then, zinc interacts with the sulfur in the substrate or in precursors of VSC, forming insoluble sulfides since it has an affinity for sulfur and oxidizes sulfhydryl groups (SH) as well as it prevents the oxidation of SH groups to disulfide bonds (225).

Chlorine dioxide is a potent oxidizing agent and a free radical with known bactericidal, viricidal and fungicidal properties. Its antimicrobial activity may be achieved by inhibiting protein synthesis through oxidizing methionine and removing it from a triplet of bacterial messenger RNA consequently leads to the cell lysis and death. It

inhibits the microbial growth by disruption of the transport of nutrients across the cell membrane. Also, it elevates the oxygen tension in both saliva and plaque, removes residual organic solutes, and suppresses the activity of bacterial proteolytic enzymes (34, 40). ClO_2 mouth rinse kills oral bacteria associated with the development and/or progression of oral diseases up to 99% in 10 seconds, and it is less toxic than CHX to human gingival cells *in vitro* (34, 57, 226-228). The oxidative load placed on the cells by the action of chlorine dioxide mean that most microorganisms are unable to build up resistance to ClO_2 (229).

An ideal prerequisite for a successful antimicrobial agent is that all bacteria within the biofilm should be exposed at an adequate concentration for an adequate time to achieve a clinically relevant reduction in pathogenesis (230). The rate and extent of antimicrobial agent penetration depend on factors including the biofilm structure and composition, biofilm thickness, as well as the physicochemical properties of the solute (231, 332). ClO_2 is a small molecule relative to common organic disinfectants such as CHX, peracetic acid, and CPC. It is also a gas, non-ionic, and soluble in water, oil, and organic solvents. These properties no doubt facilitate the transporting process through a bacterial cell wall (212). Unlike non-oxidizing disinfectants, ClO_2 kills microorganisms even when they are inactive (229). Also, the bacterial recovery after sterilization with ClO_2 is somewhat slower (233).

Aloe vera is a polysaccharide gel with antibacterial, antiinflammatory, antioxidant and healing properties. These beneficial properties are attributed to the combined action of heterogeneous chemical composition working together rather than any individual active ingredients. Some compounds in ALV gel like anthraquinones and saponin have direct antibacterial activities while some other components, such as acemannan, have been considered to exert indirect bactericidal activity through stimulation of phagocytosis (170). Additionally, it contains various antiinflammatory agents such as carboxypeptidase; which reduce prostaglandin synthesis, magnesium lactate; which inhibits histidine decarboxylase preventing mast cell activity, sterols and lupeol as pain modulators, barbolin and aloe emodin which block prostaglandin synthesis (19, 168, 170). ALV also reduces edema by inhibiting matrix metalloproteinases, blocking polymorphonuclear leucocyte (PMNs) release, cyclooxygenase, and lipoxygenase pathways. These activated PMNs, in turn, inhibit free oxygen radicals (170). The antiseptic property of ALV is due to presence of six antiseptic agents namely lupeol,

salicylic acid, urea nitrogen, cinnamonic acid, phenols and sulphur. These compounds have an inhibitory action on fungi, bacteria and viruses (175). When ALV is used at full strength at 100% concentration, it produces a maximum antibacterial effect and reduces accumulated plaque significantly (234). On the other hand, a number of clinical reports found that topical and systemic administrations of ALV gel is not effective in terms of its therapeutic activities or even it can cause undesirable effects such as retardation of wound healing. These conflicting results could be explained by the stability of the active ingredients as it was proven that the time of treatment after harvesting was an important factor that determined activity or by the use of plants from different locations with variations in their chemical composition and also because of different isolation techniques that were used to extract compounds from the aloe leaf pulp (19, 172).

In the tested formulation, zinc was utilized at low concentration to avoid its astringency and bad taste that associated with high concentration. But, its sole use in low concentration reduces its effect on plaque. A concentration ranging from 0.2-2.0% of zinc salts have demonstrated effective plaque and calculus reduction in several clinical studies either when used alone or in combination with antimicrobial agents (41-43,136-137).

Xylitol is used as a natural non cariogenic sweetener in the tested mouth rinse formulation. It decreases *S. mutans* counts, amount of plaque and the incidence of dental caries by increasing the salivary flow and pH (44). Also, the blend of citrus and mint oils provides unique flavoring and soothing taste. It increases the salivary flow, maintains the moisture and the balance of oral environment and tissue (200).

Unlike CHX, the substantivity of ClO_2 in saliva is 9 hours (31). Lang and Brecx (126) stated that the substantivity of an antimicrobial agent needs sufficient contact time with microorganisms in order to inhibit or kill it. Also, formulation of 0.1% stabilized ClO_2 with 0.05% sodium fluoride NaF demonstrated 8 hours duration of action. But, when the same concentration of ClO_2 combined with 230 ppm fluoride monoflourophosphate the duration of action increased to 12 hours. Similarly, another combinations contain ClO_2 with xylitol, peppermint oil, and citrus lemon peel oil or ClO_2 with green tea, ALV, tea tree oil, xylitol, and zinc gluconate, have an improved duration of action over 12 hours compared with 8 hours of the sole ClO_2 rinse (235-238). This can be attributed to the combined effect of many active ingredients. It was reported by Saini (224) that Zinc with

xylitol further prevents the colonization of initial plaque formation and removes halitosis-causing volatile organic compounds and can prolong the action of ClO_2.

Despite the antiinflammatory effects of both ALV and ClO_2, gingival inflammation reduction was non-significant in all groups since the study was conducted on periodontally-healthy subjects and the four-day duration of refrained oral hygiene is not enough to assess the antigingivitis effect (124, 140). In order to figure out the antigingivitis effect of the tested formulation, long-term studies such as experimental gingivitis and home-use study models are required. Collection of the 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. Results of the present study supported the antiinflammatory effect of 0.2% CHX by GCF volume reduction. However, ALV+ClO_2 combination failed to show this effect.

As a summary, ALV+ClO_2 combination mouth rinse can target dental biofilm at different mechanisms by altering biofilm formation, echology and structure, as well as reducing the microbial adhesion. In addition to bactericidal and bacteriostatic mechanisms, it controls the nutrients by inhibiting the bacterial key enzymes, controls pH by inhibiting the acid production, and the redox potential by increasing the oxygen level. This formulation can be an optimum choice for certain selected conditions where it can improve the oral and soft tissue status, such as individuals with mucositis, xerostomia, lichen planus, oral candidiasis, immunodeficiency, head and neck radiation therapy, and chemotherapy where the use of CHX or alcohol-based mouth rinse may be irritant.

Within the limit of this study, it was concluded that a mouth rinse containing ALV, ClO_2, Zn^{+2}, xylitol, and citrus and mint oils has less plaque-inhibitory effect than that of CHX, but has better taste perception, patient acceptance and preference. Further randomized, controlled, long-term clinical home-use trials are necessary to evaluate the antiinflammatory effect of the tested formulation with microbiological and biochemical analysis.

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7. APPENDICES

Appendix (I)- Ethic Approval

Araştırmanın Açık Adı		**Aloe Vera İçerikli Ağız Gargarasının Plak İnhibisyonu Üzerine Olan Etkisinin Klinik ve Mikrobiyolojik Olarak Değerlendirilmesi**
VARSA ARAŞTIRMANIN PROTOKOL KODU		
ETİK KURUL BİLGİLERİ	ETİK KURULUN ADI	**Yeditepe Üniveristesi KAEK (2012-KAEK-70)**
	AÇIK ADRESİ:	Yeditepe Üniversitesi Hastanesi İçerenköy Mahallesi, Hastane Yolu Sokak no:102-104 34755 Ataşehir İstanbul
	TELEFON	+90 (216) 578 40 00 / 4797
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BAŞVURU BİLGİLERİ	KOORDİNATÖR/SORUMLU ARAŞTIRMACI UNVANI/ADI/SOYADI	Prof. Dr. Bahar Eren Kuru Dt. Nesrin Alnaihoum			
	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	**Aloe Vera 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:** 653	**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 k**arar 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 Broşürü**nden edinebilirsiniz. Ayrıca Hastanemiz personeli hakkında http://www.yeditepehastanesi.com.tr/ web sayfamızdan daha detaylı bilgilere ulaşabilirsiniz.
- Burada belirtilenlerden başka sorularınız varsa bunları yanıtlamak görevimizdir.

BAŞH.P.06-F.04 Rev 2, 16.04.2014

TANIMLAMA

Araştırmanın Adı / Protokol numarası

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

Araştırma Konusu

Faklı içerikli ağız gargaralarının plak oluşumunu önleyici etkinliklerinin değerlendirilmesi.

Araştırmaya Katılımcı Sayısı

33

Bu araştırmanın

Amacı

Bu çalışmada, farklı içerikli ağız gargaralarının, gönüllülerin diş fırçalamayı gerçekleştiremediği durumlarda, plak oluşumunu önleyici etkinliklerinin 4 günlük plak akümülasyon modeli ile klinik ve biyokimyasal olarak değerlendirilmesi amaçlanmaktadır.

Süresi

10 hafta

İzlenecek Yöntem / Yöntemler

Bu çalışma randomize kotrollü, çift kör , cross-over, 4 gün plak akümülasyon modeli klinik ve biyokimyasal çalışmadır.

Çalışmamıza Yeditepe Üniversitesi Diş Hekimliği Fakültesi'ne rutin ağız ve diş sağlığı kontrolleri için başvuran, sistemik ve periodontal açıdan sağlıklı gönüllü bireyler dahil edilmesi planlanmaktadır. Planlanmış örneklem büyüklüğü dahilinde 18- 25 yaş arası 33 gönüllü dahil edilecektir.

Onamları alınan ve çalışmaya dahil edilme kriterlerine uygun Gönüllülerin herbiri 1, 2,3,..... 33 numaraları ile kodlanacaktır.

Gönüllüler ve araştırmadan sorumlu hekim kullanılan gargara şişelerinde hangi gargara olduğunu bilmeyecektir ve önceden hazırlanmış kapalı zarflar ile kendilerine atanacaktır.

Kodlanan hastalar 3 ayrı sıralamaya sahip 3 ayrı gruba randomize edilecektir.

Gönüllülerin herbirine, çalışmaya başlanması planlanan tariten 1 hafta öncesinde supragingival profilaksi ve polisaj işlemleri yapılacak, oral hijyen eğitimi ve çalışma periyotlarında kullanmaları için herbiri aynı olmak üzere diş fırçası ve diş macunu verilecektir.
Herbir periyodun ilk gününde (0.gün) supragingival profilaksi ve polisaj işlemlerini takiben, Dişeti oluğu sıvısı, herbir kadrandaki 1 dişin dişeti cebi içerisine 30 sn süreyle yerleştirilen periopaper yardımıyla alınacak ve PERİOTRON 8000 cihazı yardımıyla hacimsel değeri ölçülecektir.
GI ölçümleri sonrasında PI skorları plağın diş yüzeyinde görülebilir olmasını sağlayan allerjen olmayan bazik Fuksin solüsyonunu dişlerin bukkal/palatinal-vestibül/lingual yüzeylerine uygulanarak gözlemsel olarak kaydedilecektir. Çalışmada kullanılacak herbir gargara aynı şişe özelliklerinde olup, hem ölçüm yapan çalışmadan bağımsız hekim hemde gönüllü gargaranın içeriklerini bilmeyecektir. Böylece çalışmanın çift-körlülüğü sağlanacaktır. Ölçümlerin skorlanmasından sonra, gönüllü gargarayı nasıl kullanacağı konusunda bilgilendirilecek ve 4 gün boyunca kendisine atanan gargara haricinde hiç bir mekanik temizlik yapmayacağı, arayüz temizleyici ajanlar kullanmayacağı anlatılacaktır. Gargaraların kullanımı sabah 20 ml 30 saniye kahvaltıdan sonra ve akşam yatmadan once 20ml-30 saniye şeklinde olacaktır. Gargaraların kullanımından sonra gönüllüler 30 dakika hiç bir yiyecek içecek tüketilmemesi söylenecektir. Klinik ölçümler sonunda gönüllülere supragingival profilaksi ve polisaj işlemleri yapılacaktırr.
4 gün gargara kullanımı sonunda gönüllülerden DOS örnekleri ve PI GI skorlamaları yapılacaktır. Bu 4 günlük period sonunda hastalar anınma dönemi olarak adlandırılan wash-out periyoduna girecek ve bu periyodda kendi diş fırçalama ve arayüz temizliği alışkanlıklarına devam edecektir.

Araştırma Sonunda Beklenen Fayda
Hataların diş fırçalamayı gerçekleştirmediği durumlarda, periodontal hastalık oluşumunda primer etyolojik faktör olan mikrobiyal dental plağın diş ve çevre dokularda oluşumunun önlenmesi beklenmektedir.

Alternatif Tedavi Veya Girişimler

Araştırma Sırasında Karşılaşılabilecek; bildirilmiş herhangi bir risk yoktur.

Riskleri	*Rahatsızlıklar*
a)	a) Kötü tat hissi
b)	b)
c)	c)
d)	d)
e)	e)

f) g)	f) g)

Risk / rahatsızlık durumlarında yapılması gerekenler
Rahatsızlık durumunda lütfen araştırma sorumlusu ile iletişime geçiniz.

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.

Bu çalışma bir DOKTORA TEZİ araştırmasıdır.

Araştırmada uygulanacak tedaviler

Sistemik ve periodontal açıdan sağlıklı Yeditepe Üniversitesine rutin diş sağlığı kontrolleri için başvuran gönüllü katılımcılar araştırmaya başlanması planlanan tarihten 10 gün önce araştırmaya dahil edilme kriterleri doğrultusunda çalışmaya dahil edilecek ve 3 farklı çalışma grubuna bilgisayar programı kullanılarak randomize edilecektir. Bu üç farklı grup 3 farklı gargarayı Latin square cross over dizaynı doğrultusunda kullanacaktır.

Randomizasyon tablosu aşağıdaki gibidir.

	PERİOD 1 4 gün	Arınma dönemi	PERİOD 2 4 gün	Arınma dönemi	PERİOD 3 4 gün
GROUP 1 N=11	A gargarası	10 gün ⟷	B gargarası	10 gün ⟷	C gargarası
GROUP 2 N=11	B gargarası	10 gün ⟷	C gargarası	10 gün ⟷	A gargarası
GROUP 3 N=11	C gargarası	10 gün ⟷	A gargarası	10 gün ⟷	B gargarası

Bireylerin gruplara randomizasyonu bilgisayar programı yardımı ile yapılmıştır.

Herbir grup (n=11), çalışmanın her bir Periyodunda farklı içerikli gargaralar ile araştırmaya başlayacaktır.

Örnek: Grup 1 n=11 1. Periodda A içerikli gargarayı
2. periyodda B içerikli gargarayı
3. periyodda C içerikli gargarayı kullanacaktır.

Grup 2 n=11 1. Periodda B içerikli gargarayı
2. periyodda C içerikli gargarayı
3. periyodda A içerikli gargarayı kullanacaktır.

Grup 3 n=11 1. Periodda C içerikli gargarayı
2. periyodda A içerikli gargarayı
3. periyodda B içerikli gargarayı kullanacaktır.

Her bir periyot 4 günlük gargara kullanımını ifade etmektedir. Bu dönemde gönüllü katılımcılar kendilerine atanan gargaradan başka hiçbir mekanik temizleyici diş fırçası ve arayüz temizleyici fırça veya diş ipi kullanmayacaklardır. Periyotlar arasında 10 gün arınma dönemi uygulanacaktır. Arınma dönemlerinde katılımcılar kendi ağız hijyen bakımlarını uygulayacaktır.

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

Bilgilendirilmiş Gönüllü Onam Formu asgari olarak yukarıda belirtilen başlıkları içermelidir.

Appendix (III)- The Written Instruction Form

Dear participant, we would like you to carefully read and follow the following instructions :

1. During the pre-experimental week you have to brush your teeth twice a day for not less than 2 minute using the standard manual toothbrush along with the standard toothpaste. In addition, you have to use the standard dental floss for cleaning interdental areas but **NO** any chemical agent containing antimicrobials, in a form as mouth rinses, sprays, powder, gel, chewing gums, and lozenges, can be used.

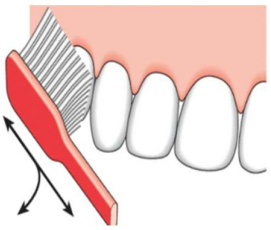
2. Throughout the whole experimental period and during every four days of each treatment period, you have to stop all the oral hygiene care measures including tooth brushing and flossing and only use the given mouth rinse twice daily for the four day period as the following:

   - 10 ml must be measured with the provided measuring cup.
   - Keep in your mouth for 60 seconds with the help of stop watch.
   - Spit it without subsequent rinsing with water .
   - Please stay without drinking, eating or rinsing for 30 minutes.
   - You have to stop all the oral hygiene care measures **EXCEPT** the given mouth rinse.

3. During the rest period (Washout period) of 10 days between the treatment periods you have to return to the oral hygiene care measures including tooth brushing and flossing **ONLY**.

Tooth Brushing

You have to brush twice daily for at least 2 minutes using the methods as illustrated below

	Place bristles along the gumline at 45˚ angle and the bristles should contact both tooth surface and the gumline.
	Gently brush the outer surfaces of 2-3 teeth using a vibrating back, forth and rolling motion and then sweep the bristles over the crown of the tooth, toward biting surface of the tooth. Move the brush to the next group of 2-3teeth and go in sequence 
	Brusth the inner surfaces of teeth by placing bristles along the gumline at 45˚ angle and the bristles should contact both tooth surface and the gumline and make a vibrating back, forth and rolling motion and then sweep the bristles over the crown of the tooth, toward biting surface of the tooth.
	Brush the inner surfaces of anterior teeth by placing the brush vertically make several up and down strokes with the front half of the brush and then sweep the bristles over the crown of the tooth, toward biting surface of the tooth
	Place the brush against the grinding surfaces of the teeth with back and forth scrubbing motion

Interdental Cleaning - Flossing

For cleaning the interdental spaces where manual brush can not clean you have to use the dental floss once a day and you have to use it as it illustrated in the box below

	Take 18″of floss and wind it around the middle finger of each hand. Hold the floss tightly between your thumb and forefingers.
	Keep 1-2″ length of floss taut between fingers. Use index finger to guide the floss between the lower teeth.
	Gently guide the floss between your teeth using gentle sawing or back and forth motion ,
	Slide the floss up and down against the tooth surface and under the gumline. Floss each tooth throughly with a clean section of the floss. Repeat this method for rest of the teeth. Don't forget the back side of your last teeth.

- *Please do not hesitate to get in touch if there is any doubts, we will clarify them.*
- *Please do not hesitate to inform us if there is any adverse effect happened during the use of the given mouthwash even you feel it could be neglected.*

Thank you for participation

Appendix (IV)- The Clinical Assessment Form

Clinical Assessment form

Gp1

Patient name&Surname : , **DOB:** / /19

Gender: , **Telephone No :** , **GCF Sampling Code : 1. 1 . 1**

Treatment period Sequence : A- B- C **Product of Perference: A, B, C**

Checklist :

DAY	Fri	Fri	Tues	Fri	Tues	Fri	Tues
TREATMENT PERİOD	D-7	D0-tx1	D4-tx1	D0-tx2	D4-tx2	D0-tx3	D4-tx3
DATE							
SGS&P							
Interproximal Flossing							
Cleaning of distal surface of last molar							
GCF Sampling							
GI							
PI							
Questionnaire							
Complianc							
Oral&soft Tissues Status							
Adverse Event							

Clinical Parameters:

I - GCF Volume :

Treatment Day - tx	GCF Volume (µl)	Tooth No.	Side	GCF Volume (µl)	Tooth No.	Side	GCF Volume (µl)	Tooth No.	Side	GCF Volume (µl)	Tooth No.	Side
D0- tx1												
D4- tx1												
D0-tx2												
D4-tx2												
D0-tx3												
D4-tx3												

II- Modified Gingival Index (Lobene)

Upper Jaw

F	4 day-3														
	0 day-3														
	4 day-2														
	0 day-2														
	4 day-1														
	0 day-1														
		17	16	15	14	13	12	11	21	22	23	24	25	26	27	Total
P	0 day-1															
	4 day-1															
	0 day-2															
	4 day-2															
	0 day-3															
	4 day-3															

Lower Jaw

L	4 day-3														
	0 day-3														
	4 day-2														
	0 day-2														
	4 day-1														
	0 day-1														
		47	46	45	44	43	42	41	31	32	33	34	35	36	37	Total
F	0 day-1															
	4 day-1															
	0 day-2															
	4 day-2															
	0 day-3															
	4 day-3															

Index	Total score / The number surfaces examined	Mean Index
D0-tx1		
D4-tx1		
D0-tx2		
D4-tx2		
D0-tx3		
D4-tx3		

III- Plaque Index (Tureskey Modification of Quigely Hein Index)

Upper Jaw

F	4 day-3														
	0 day-3														
	4 day-2														
	0 day-2														
	4 day-1														
	0 day-1														
		17	16	15	14	13	12	11	21	22	23	24	25	26	27	Total
P	0 day-1															
	4 day-1															
	0 day-2															
	4 day-2															
	0 day-3															
	4 day-3															

Lower Jaw

L	4 day-3														
	0 day-3														
	4 day-2														
	0 day-2														
	4 day-1														
	0 day-1														
		47	46	45	44	43	42	41	31	32	33	34	35	36	37	Total
F	0 day-1															
	4 day-1															
	0 day-2															
	4 day-2															
	0 day-3															
	4 day-3															

Index	Total score / The number surfaces examined	Mean Index
0 day-1		
4 day-1		
0 day-2		
4 day-2		
0 day-3		
4 day-3		

Adverse Effect

tx no	Type	Onset	severity	duration	Precipitatin factors
tx1					
tx2					
tx3					

Compliance :

Remained amount	
Used amount	
Compliance	☐ **Positive** ☐ **Negative**

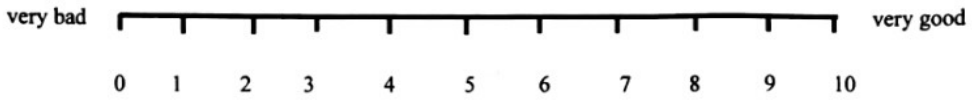
Appendix (V) - Satisfaction Questionnaire Form

Mouthrinse Questionnaire

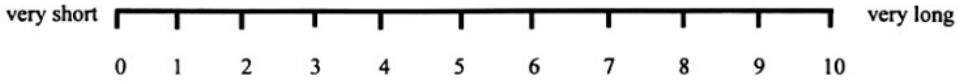
Name&Surname : **Group No : 1**

No. of Treatment Period (tx) : tx2 **Mouthwash code : MW2**

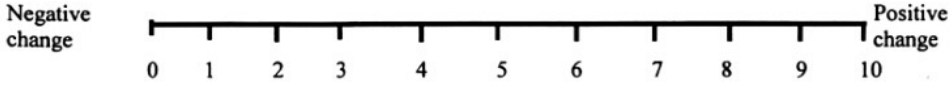
1. How was the taste of the product? ☐



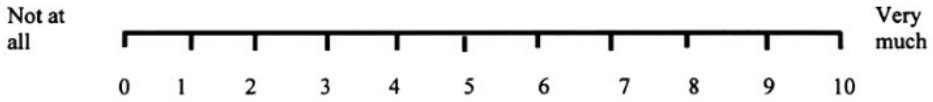
2. How long did the taste remain? ☐



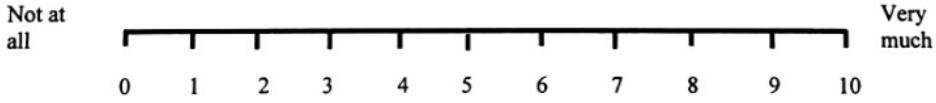
3. How was your taste of food and drinks affected? ☐



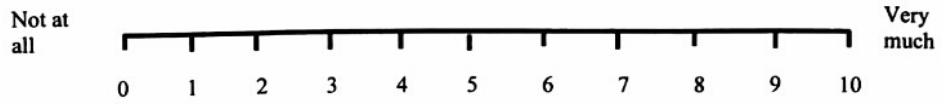
4. Did you experience sensitivity in your mouth and / or the teeth because of the mouthwash? ☐



5. Did you experience a burning sensation in the mouth because of the mouthwash? ☐

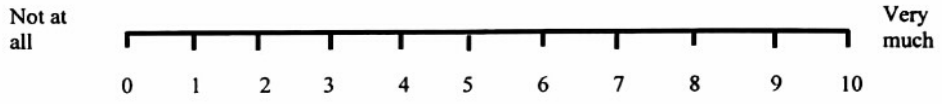


6. Did you experience a dry mouth because of the mouthwash? ☐

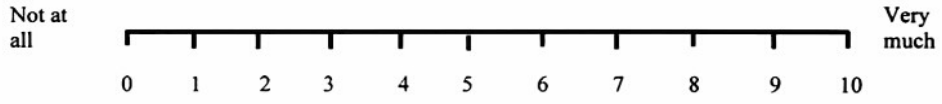


7. Did you experience a numbness feeling in the mouth because of the mouthwash? ☐

8. Did you experience staining on the teeth because of the mouthwash? ☐



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



8. CURRICULUM VITAE

Name	**Nesrin**	**Surname**	**EL-NAIHOUM**
Place of birth	**27/05/1980**	**Date of birth**	**Benghazi-LIBYA**
Nationality	**Libyan**	**TC Identification Number**	**99994347116**
E-mail	**nessrinalnaihoum@ymail.com**	**Tel.**	**05436334036**

Education degree	**Area**	**Graduated Name of Organization**	**Graduation year**
Doctorate			
Bachelors	**Oral and Dental Surgery**	**Beghazi University**	**2004**
High school	**-**	**El-Saida Roquia School**	**1998**

Languages	**Foreign exams note**
Arabic	**Mother Tongue**
English	**ILETS Total Score 6**

Task	**Institution**	**Time**
Demonstrator	**Faculty of Dentistry, Benghazi University**	**2008-2010**
General Practitioner	**Central Dental Clinic OF Benghazi**	**2007-2008**
General Practitioner	**Dubi Dental Center**	**2006-2007**
General Practitioner	**7 th October Hospital**	**2004-2007**

Computer Information Program	**Using skills**
International Computer Driving Licence	**good**