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Efficacy of a newly formulated foam on gingival inflammation: a pilot study

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EFFICACY OF A NEWLY FORMULATED FOAM ON GINGIVAL INFLAMMATION: A PILOT STUDY

by

PRIYA GUPTA VAJRAPU

Madha Dental College & Hospital, 2013

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Approved by

First Reader
Janice Weinberg, Sc.D.
Professor of Biostatistics, Public Health
Director, Master of Science in Clinical Investigation,

Second Reader
Hatice Hasturk, D.D.S., Ph.D.
Associate Member of the Staff
Director, Center for Clinical and Translational Research Center
Adjunct Associate Professor, Department of Molecular and Cellular Biology
Goldman School of Dental Medicine

Third Reader
Theresa A. Davies, Ph.D.
Assistant Professor of Medical Sciences & Education
DEDICATION

I would like to dedicate this work to my grandmother Kukutla Yamuna. Hopefully through the intense study of Periodontal disease better treatments will become available to patients in future.
ACKNOWLEDGMENTS

I would like to thank my committee members for their support and guidance through the thesis process, as well as for their assistance with editing. I would also like to thank my mother Kukutla Rajalaxmi for her support and editing assistance.
EFFICACY OF A NEWLY FORMULATED FOAM ON GINGIVAL INFLAMMATION-A PILOT STUDY

PRIYA GUPTA VAJRAPU

ABSTRACT

Periodontal disease, gingivitis and periodontitis are conditions that are a result of local response to supragingival dental plaque that forms due to poor personal oral hygiene. This is initiated by accumulation of bacterial biofilm on the teeth that leads to inflammatory changes in the gingival tissue. The pathogenesis of periodontitis has a multi-level architecture, composed of bacterial composition, environmental and genetic factors. Disruption of the oral biofilm by mechanical methods is one of the best alternatives for preventing periodontal disease.

The present intervention study aimed at decreasing the gingival inflammation in 36 patients with gingivitis or mild to moderate periodontitis by administration of a new dental product composed of antioxidants (dental foam). This study was conducted as a proof-of-concept study over 42 days and aimed to observe the earliest changes in gingival inflammation as measured by gingival index and bleeding on probing. Clinical periodontal parameters including gingival index, bleeding on probing, plaque index and probing pocket depth were assessed at baseline, and 14, 28 and 42 days after baseline. Subjects in both treatment (n=24) and control (n=12) groups were given standard oral hygiene instructions including brushing with a standard toothbrush (Oral B® Pro health vi
medium) and standard toothpaste (GLO Science Toothpaste) twice a day. Subjects in test group used the dental foam in addition to the standard toothpaste twice a day, while control group subjects did not use any additional product.

The statistical analyses were performed to compare the mean changes from baseline to each post baseline time points using Student’s t-test. All statistical tests were conducted at \( p< 0.05 \) level of significance. There was a statistically significant reduction in the primary endpoints, gingival index \( (p=0.003) \) and bleeding on probing \( (p=0.007) \) in the test group when compared to the control group over 42 days. There were no statistically significant differences in the secondary outcomes, plaque index \( (p=0.07) \) and pocket depth \( (p= 0.12) \) between two groups. Oral hygiene care including mechanical plaque removal with standard tooth brushing in combination with application of newly developed dental product (dental foam) has shown significant reduction in gingival inflammation when compared to standard tooth brushing alone and reveals beneficial effects in patients with gingivitis and mild to moderate periodontitis.
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LIST OF ABBREVIATIONS

AAP .............................................................................................................. American Academy of Periodontology
Aa..................................................Aggregatibacter (Actinomyces)Actinomycetemcomitans
BOP ............................................................................................................. Bleeding on probing
Bf.................................................................Bacteriodes Forsythus
CAL ................................................................................................. Clinical Attachment Level
CDC .................................................................................. Center for Disease Control and Prevention
CEJ ......................................................................................................... Cementoenamel junction
FIRB.............................................................. Forsyth Institutional Review Board
GCF ..................................................................................................... Gingival Crevicular Fluid
GI ........................................................................................................ Gingival Index
IL ........................................................................................................ Interleukin
LPS........................................................................................................ Lipopolysaccharides
MMP .................................................................................................. Matrix metalloproteinase
NIDCR ................................................ National Institute of Dental and Craniofacial Research
PD ........................................................................................................ Pocket depth
PDI ...................................................................................................... Periodontal Disease Index
PG .................................................................................................... Prostaglandins
Pg.............................................................. Porphyromonas Gingivalis
PI ........................................................................................................ Plaque Index
Pi ........................................................................................................ Prevotella Intermedia
PPD ................................................................. Probing pocket depth
SRP .............................................................. scaling and root planning
TNF-a .............................................................. tumor necrosis factor alpha
INTRODUCTION:

The term gingivitis refers to inflammation of gingiva, and commonly occurs due to accumulation of biofilm containing bacteria on teeth. Gingivitis is a reversible type of periodontal disease, however if left untreated, gingivitis can progress to periodontitis, which is more advanced and can eventually lead to loss of teeth (Miller, 2012). Periodontitis is a prevalent disease with a pathological process, which leads to inflammation of the supporting tissues of the teeth including gingiva, periodontal ligament, alveolar bone and cementum (Figure 1). The progressive extension of gingival inflammation into the adjacent bone and periodontal ligament leads to destruction and eventually loss of periodontal ligament and alveolar bone (The American Academy of Periodontology, 2001).

Gingivitis is initiated by bacteria in the biofilm that causes pathological changes in the tissues directly or indirectly. The disease is limited to the gingival epithelium and connective tissue. Clinically, gingivitis is characterized by gingival redness, edema, bleeding, changes in contour, and increased in the volume of the gingival crevicular fluid (GCF) (Arul et al., 2014). Periodontitis is clinically differentiated from gingivitis by the loss of connective tissue attachment and alveolar bone supporting teeth in the presence of progressive gingival inflammation.

Both clinical and radiological evaluations are required to assess the severity and extent of the periodontal disease in the clinical setting. Clinically, probing pocket depth and clinical attachment level determines the extent and the severity of the disease. Radiographically, the amount of crestal (horizontal) and vertical bone loss in the
interproximal areas, width of the periodontal ligament space and bone loss in furcation areas depicts the severity of the disease. As the lesion progresses the bone loss also progresses apically (Anbiaee & Tafakhori, 2012).

![Figure 1 Anatomy of the Tooth.](Pandit, 2016)

**Classification of periodontal disease:**

Several classifications have been used to categorize types of periodontal disease. Although many different classifications with different clinical manifestations of
periodontitis have been presented over 20 years, the 1989 American Academy of Periodontology (AAP) world workshop in clinical periodontics identified that periodontitis exists in several stages early onset, adult onset and necrotizing forms. Based on this AAP created the periodontal classification system (Figure 2) (Armitage, 2004).

Figure 2 Classification of Periodontitis.
Shown are the five stages of periodontal disease (Armitage, 2004)

In addition to the periodontal disease classification from the 1989 AAP world workshop, the European AAP workshop presented an alternate classification system in 1999 (Armitage, 1999). The European AAP found that there was not much supporting evidence to support the refractory stage of periodontitis. Moreover, the classification was not consistent enough to be applied to population in different countries and also it did not fit all models presented during the workshop. Hence the resulting classification was based on the scientific evidence and was presented by AAP international workshop in 1999 (Figure 3).
Figure 3: Classification of Periodontitis
Modifications to the 1989 AAP classification system (Armitage, 1999).

Periodontitis is further sub-classified into the above mentioned categories based on the clinical, radiographic, historical, and laboratory characteristics. Chronic periodontitis is the most common form of periodontitis seen in adults but is also present in children as well. Chronic periodontitis is usually associated with the accumulation of plaque and calculus with, the disease progression rate varying from slow to moderate. The disease is described by the severity as slight, moderate or severe based on the amount of clinical attachment loss (Flemming, 1999). Slight periodontitis is defined as the amount of periodontal destruction ≤2 mm of clinical attachment loss (CAL), while sites with moderate periodontitis show 3-4 mm of CAL. Severe periodontitis is associated with >4 mm of CAL. Chronic periodontitis is sub classified into generalized and localized form based on the sites involved.

Aggressive periodontitis differs from the chronic form by the rapid progression of the disease state, absence of accumulation of plaque and calculus, and familial history of
the aggressive disease (Novak & KF, 1996). This form of periodontitis is classified as early – onset periodontitis which was formerly sub-classified as pre pubertal, juvenile and rapidly progressive forms with localized or generalized disease distribution (Tonetti & Mombelli, 1999). Lately aggressive periodontitis is classified as localized and generalized.

Periodontitis as a manifestation of systemic diseases is usually associated with the hematologic and genetic disorders of affected individual (Kinane, 1999). The majority of these disorders occur due to the alterations in the host defense mechanisms. The clinical manifestations of these disorders appear at an early age and may be mixed up with the aggressive form of periodontitis with rapid attachment loss and potential for early tooth loss. Presently, periodontitis, as a manifestation of systemic diseases, is diagnosed when the systemic condition is the major predisposing factor and local factors such as large quantities of plaque and calculus are not clearly evident (Kinane, 1999).

A causal relationship has been established between oral hygiene and gingivitis. The microbial flora in deep pockets of subgingival margin effects personal hygiene practices of individuals. Good oral hygiene can be achieved though removal of plaque by tooth brushing, flossing and professional cleaning, yet there are number of people who do not have sufficient dexterity to achieve the desired oral hygiene (Van Dyke et al., 1999).

**Epidemiology of Periodontal Disease:**

The National Institute of Dental and Craniofacial Research (NIDCR) reported that gingivitis is more severe and prevalent in adolescence when compared to early childhood and older age groups. Over 50% of adults had gingivitis on an average of 3 to 4 teeth and
subgingival calculus was present in 67% population (NICDR, 2014). According to a report by Center for Disease Control and Prevention (CDC), 47.2% adults aged 30 years and older have some form of periodontal disease (Eke et al., 2012). (Eke, et al., 2012). Disease progression increases with age and rises to 70.1% in adults 65 years and older. This condition is more common in men accounting for 56.4% than women who accounted for 38.4% of periodontitis (CDC, 2013).

Among people affected with severe periodontitis a significant majority (65.4%) was found in individuals living below the federal poverty level while those with less than a high school education constituted 66.9%, and current smokers accounted for 64.2% (CDC, 2015). It has been documented that only 5% to 15 % of any population suffers from severe generalized periodontitis even though the majority of the population is affected by the disease (Oliver et al., 1998).

Many surveys have assessed the periodontal status in the U.S. population. The most popular of these surveys are the National Health and Nutrition Examination Survey 1971-1974 that assessed the periodontal status visually. Later NHANES III 1998-1994 and NHANES 1999-2004 assessed the periodontal status using the partial mouth periodontal examination (PMPE). (Eke et al., 2012). Only two sites per tooth were assessed for pocket depth (PD) and clinical attachment loss during NHANES III and NHANES 1999-2000. This assessment further involved three sites per tooth using the PMPE at NHANES 2001-2004. (Eke et al., 2010). After all these challenges and interpretations, it was suggested that in terms of pocket depth and attachment levels there has been an improvement in periodontal disease prevalence according to the NHANES survey data of 1998-2004 (Dye,
et al., 2007). Furthermore, full mouth periodontal examination (FMPE) began during NHANES 2009-2010, probing from six sites per tooth to establish true data on the prevalence of periodontitis in the United States.

According to European Federation of Periodontology and other published studies (Eugenio et al., 2015), two or more interproximal sites with ≥ 6mm clinical attachment loss or one interproximal site with ≥ 5 mm CAL is defined as severe periodontitis. Whereas, two or more interproximal sites with CAL ≥ 4 mm or 5 mm pocket depth (PD) is classified as moderate periodontitis and two or more interproximal sites with ≥ 3 mm CAL or with ≥ 4 mm PD is defined as mild periodontitis (James et al., 2001). In NHANES 2009-2010, severity of PD and CAL were reported using measurements from all the six sites. The mean and prevalence of CAL and PD ranged from 3 mm to 7 mm. the extent of disease was reported as 5, 10, and 30% of sites and teeth (Tonetti & Claffey, 2005).

**Etiology of Periodontal Disease:**

Gingivitis and periodontitis are inflammatory conditions of infectious nature (Williams, 1990). The disease has different stages ranging from mild gingivitis to irreversible and advanced conditions, including severe periodontitis (Jemin & Salomon, 2006). Apart from pathogenic microorganisms, genetic and environmental factors also contribute to the development of periodontal disease. Earlier it was thought that periodontal disease occurred in response to plaque mass (non-specific plaque hypothesis), but later the specific microbial species were shown to be responsible of disease initiation and
progression (specific plaque hypothesis) (Loesche & Grossman, 2001). Formerly, the disease occurs in a site already colonized by a bacterial population such as the exogenous pathogens which include Capnocytophaga spp and Prevotella spp. More recently, additional studies have been conducted to support that there are unidentified and uncultivable microbial species that are essential for disease initiation and progression (Mario et al., 2008). The rate of disease progression at the sites with baseline attachment loss is not always the same at all sites and time points (Goodson et al., 1982).

One of the most significant developments in the etiology of periodontal disease is the role of dental plaque as a biofilm. Microbial plaque is the primary etiological factor in chronic inflammatory periodontal disease. The hard and soft tissues of the oral cavity such as masticatory mucosa, dorsum of the tongue, saliva, tooth surfaces and restorative materials are areas where bacteria colonize and create different ecosystems with different bacterial profiles. The bacteria adhere to these structures and form a matrix called “biofilm” that consists of mixed population of pathogens (Kolenbrander, 2000). The biofilm allows the microorganisms to unite and multiply on different surfaces (Rita, et al., 2011). It also protects the microorganisms from toxic substances in the environment and aids in the uptake of nutrients creating a suitable environment for their growth (Socransky & Haffajee, 2002).

There are five major bacterial complexes classified by Socransky and Haffajee based on varying virulence. Some of the major gram negative anaerobes classified under red complex bacteria’s present at the diseased sites are Aggregatibacter (formerly known as Actinomyces) actinomycetemcomitans (Aa), Tannerella forsythensis (formerly known as
Bacteriodes forsythus), Porphyromonas gingivalis (Pg), Prevotella Intermedia (Pi), Fusobacterium nucleatum, Campylobacter rectus and Treponema denticola. (Socransky & Haffajee, 2002). The clones of these bacteria are closely associated with periodontitis and are found to be pathogenic. The supragingival plaque serves as a natural reservoir and bacteria in supragingival plaque migrates subgingivally to form a subgingival biofilm if supragingival plaque persists as conditions allow.

Certain genetic factors are considered as risk factors for periodontal disease. For example, specific genotype polymorphic IL-1 gene cluster is one of the factors responsible for severe periodontitis. The pro inflammatory cytokine Interleukin (IL)-1 is a key regulator of the host response to a microbial infection especially in patients with history of smoking. Genotype IL-1 positive patients were found to have higher mean counts of individual virulent bacterial species in pockets deeper than 6mm, including Tanerella Forsythia (T. forsythia), Porphyromonas gingivalis (P. gingivalis) and Treponema denticola (T. denticola) (Samuel, et al., 2008). In addition, macrophages are in a hyper immune state, producing increased amounts of tumor necrosis factor- alpha (TNF- α), one of the major pro inflammatory cytokines that plays a role in gingivitis and leads to early and rapid bone loss in these individuals (Narayanan & Sonika, 2011).

Environmental Factors:

The association between periodontal disease, race and ethnicity is significantly attenuated when effects by confounders such as cigarette smoking and income are accounted for (Hyman & Reid, 2003). The increased risk of periodontitis in certain
racial/ethnic groups, such as African Americans, or Native Americans, may be partly attributed to socioeconomic, behavioral and other conditions. On the other hand, there is evidence that increased risk may also be partly related to biologic/genetic predisposition (Poulton, et al., 2002).

The severity of periodontal disease can be attributed to factors such as poor oral hygiene, smoking and diabetes mellitus. Gingivitis and periodontitis are the inflammatory diseases of gingiva and supporting tissues, where the inflamed surface acts as the port of entry for various microbial pathogens (Peter, et al., 2009). The amount of bacterial pathogens present is varied between $10^8$-$10^{11}$ per mg of dental plaque, which may be sufficient to cause bacteremia when exposed to circulation and systemically spread bacterial products. These inflamed surfaces are caused by poor oral hygiene that leads to accumulation of plaque and calculus around the teeth. This in turn progressively leads to tooth loss by destructing the junctional epithelium and eventually leading to periodontal pocket formation. Similarly, there are many mechanisms through which the oral bacteria cause systemic diseases by direct or indirect effects (Xiaojing et al., 2000).

Diabetes mellitus is such a systemic disease and is associated with an increased risk of tooth attachment loss. In these association studies, diabetic parameters examined included glycemic control, duration of disease, presence of other diabetes-associated complications and the population studied. Periodontal parameters include gingivitis, clinical attachment loss, and alveolar bone loss (Tomar & Asma, 2000). Studies have shown a relationship between poor glycemic control and periodontal disease suggesting
that poorly controlled diabetics respond less successfully to periodontal therapy compared to well-controlled and non-diabetics subjects (Van Dyke & Sheilesh, 2005).

Apart from systemic diseases, periodontal disease serves as a risk factor for other inflammatory diseases such as cardiovascular disease, diabetes mellitus, preterm birth and pulmonary diseases. A study suggested that the rate of coronary heart disease increased by 25% in subjects with periodontitis when compared to periodontitis free- individuals (DeStefano et al., 1993). Moreover, 70% of men with periodontitis younger than 50 years of age had an increased risk of coronary heart disease than men without periodontal disease. Adjusted odds ratio for preterm birth or low birth weight in women with severe periodontal disease was found as 7.5 in a study with 124 mothers. Attributable risk analysis indicated that as much as 18% of all preterm birth or low birth weight cases could be due to periodontal infections (Collins, et al., 1994).

**Pathogenesis of Periodontal Disease:**

The main goal in the treatment of periodontal disease is to eliminate the bacterial pathogens and contributing risk factors so that disease progression can be halted and the health of the periodontium can be preserved (Jemin & Salomon, 2006). The progressive gingival inflammation leads to loss of connective tissue attachment to the teeth which serves as a basis for clinically differentiating periodontitis from gingivitis (Listgarten, 1986). Periodontitis leads to breakage in the lining of the periodontal ligament, disruption of its attachment to cementum as well as resorption of the alveolar bone. Apart from loss
of attachment there is migration of epithelial cells along the root surface causing resorption of alveolar bone (Jemin & Salomon, 2006).

The inflammatory response in periodontal disease includes the activation of leukocytes, neutrophils, T-lymphocytes and plasma cells (Figure 4). They also release antibodies, lipopolysaccharides (LPS) and chemical inflammatory mediators. The lipopolysaccharides present in the gram-negative bacterial cell walls act as powerful stimulants for the complex host response. Cytokines, chemokines and C-reactive protein are some of the chemical mediators released during the inflammatory process. Apart from these chemical mediators, other mediators such as TNF-α, interleukin-1 (IL-1) and prostaglandins (e.g., prostaglandin E₂-PGE₂) stimulates fibroblasts and secretes matrix metalloproteinases (MMPs) during the inflammatory process (Graves & Cochran, 2003). They are also responsible for the increased collagen breakdown and osteoclastic activity, which results in bone resorption increasing the destructive process.

The level of periodontal destruction depends on the balance between destructive and protective inflammatory mediators. While periodontal bacteria are required for infective periodontal disease, individual response determines disease progression (Lovegrove, 2004). Clinical models of disease activity in periodontitis range from a continuous progression of disease, during which loss of attachment occurs at a slow rate over long periods of time to an episodic burst model in which loss of attachment occurs relatively rapidly during short periods of disease activity (Jeffcoat & Reddy, 1991).
Clinical data indicate that the pathogenesis of periodontal attachment loss could differ between patients, periodontal sites and time points (Socransky et al., 1984).

**Clinical Assessment of Periodontal Disease:**

The periodontium is accepted to be normal when the pocket depth is no deeper than 3 mm, whereas in a periodontal disease state the pocket depth is above 4 mm or greater (Michael et al., 1984). One of the most commonly used indices for assessment of the status of gingival health or inflammation is gingival index (G.I) developed by Löe and Silness (Stephen & Klaus, 1981). GI was the first index to evaluate all the tooth surfaces. All buccal, mesial, distal and lingual tooth surfaces of the gingival tissue are given a score of 0-3 based on gingival redness, contour and edema.
Ramjford introduced the periodontal disease index (PDI) in 1967. He used partial recordings in his assessment of disease by examining a total of six teeth, which are now called the Ramjford teeth. He introduced the measure of CAL from the cementoenamel junction (CEJ), which serves as a fixed point. (Ramfjord, 1967). CAL is apical migration of the junctional epithelium beyond the CEJ with loss of connective tissue attachment and alveolar bone. Due to examiner variation and lack of uniformity in CAL and PD values, a number of investigators have used their own criteria to define severe periodontitis. (Haffajee & Socransky, 1986)(Hugoson et al., 1992; Locker & Leake, 1993). To establish the definitions for periodontitis, the threshold values for CAL, PD, or both at a given site should be determined that provide evidence of periodontitis at that site.

Treatment of Periodontal Disease

Non-surgical therapy:

The traditional approach in treating periodontal disease includes nonsurgical therapy followed by a surgical therapy. Phase 1 therapy is the first step that constitutes the periodontal treatment. This is a nonsurgical therapy with the main objective to eliminate pathogens and factors responsible for gingival and periodontal diseases (American Academy of Periodontology, 2000). The microbial pathogens and contributing factors can be eliminated by the treatment of the carious teeth, removal of calculus, corrections of defective restorations, and following a daily plaque control regimen (Axelsson & Lindhe, 1974). In a phase 1 therapy the removal of plaque and calculus is controlled using mechanical aids including dental floss, scalers, curettes and ultrasonic instrumentation.
Defective restoration is re-contoured using finishing burs and diamond coated files used with hand piece, while the carious lesions are removed completely and treated with temporary or permanent restorations (Lee et al., 1971). Along with mechanical aids which play an important role in elimination of plaque, the use of appropriate antimicrobial agents and devices also serve to promote elimination of the microbial pathogens.

**Professional and Home Care with mechanical aids**

Oral measures alone seem to have limited effect on treatment of severe periodontal diseases. Therefore, mechanical aids are used that change the environment for the pathogens thereby reducing the host inflammatory response (Guthmiller & Karen, 2002). Periodontal treatment consists of debridement of the tooth and the root surfaces to reduce the bacterial pathogens that are inhabited. Maintenance of periodontal health following therapy includes life-long self-care and maintenance supplemented by professional care at varying intervals (3-6 months) (Elisabeth, 1996). Usually, oral hygiene practices consist of mechanical therapy, oral hygiene instructions and maintenance.

Mechanical therapy includes the removal of supra and subgingival plaque and calculus by a professional procedure called scaling and root planning (Rajesh et al., 2011). Scaling and root planning is usually done using hand instruments or ultrasonic instruments followed by polishing using rubber cup and fluoride prophyl paste. In maintenance therapy, disclosing agents are used to visualize plaque content on the teeth in order to administer the subject with various oral hygiene instructions. Oral hygiene instructions comprise of appropriate brushing technique, interdental devices including interdental brush and dental floss and fluoride applications during dental visits. These oral hygiene instructions are
given to achieve optimal plaque control by self-care. (Silvinha et al., 2010). Mechanical supragingival plaque control by self-care is the most important component in maintenance therapy (Rajesh et al., 2011).

The success of oral care by individuals depends on several factors. Often, dexterity is found as an important factor; however, brushing technique and type of tooth brush and tooth paste used are the most important factors and are not influenced by age and dexterity. On the other hand, use of mechanical aids such as interdental brushes and dental floss are used more frequently by younger age groups than older age groups due to dissemination of information that is more effective among young generations. (Demetriou et al., 1990). Some studies have shown that only 2-10% of the population use dental floss regularly and effectively. Moreover, the compliance of its use is reduced with time even with sufficient education and motivation (Enrico et al., 2011). Taking into consideration the problems with dexterity, complexity of the interdental aids and insufficient knowledge and education in the use of mechanical aids, mouthwashes with antimicrobial agents such with phenol, chlorhexidine, and essential oils were considered convenient and effective agents. The use of antimicrobial agents supplemented with mechanical aids is known to improve the oral health maintenance.

**Periodontal treatment with surgical therapy and systemic and local antibiotics:**

The main purpose of the surgical therapy is to eliminate the pathological changes in the pocket wall and create a stable environment that could promote periodontal
regeneration. Another objective of the surgical phase is to correct the anatomic morphological defects that could potentially lead to accumulation of plaque and calculus.

The mechanical periodontal treatment alone is adequate to resolve periodontitis, but adjunctive antimicrobial regimen, delivered systemically, can enhance the effect of therapy in certain cases. Antibiotics can be administered systemically or locally. When administered systemically the antibiotics penetrate the periodontal tissues via serum (Pejčić et al., 2010). Even though the systemic delivery method has some disadvantages such as adverse drug reactions, patient compliance, antibiotic resistant micro-organisms their simple and easy mode of administration that enables to reduce the pathogens makes it more effective (Rohini et al., 2013). Systemic antibiotics along with scaling and root planning (SRP) provide ancillary benefit in the treatment of periodontitis. Surgical therapy with proper oral hygiene can prevent progression of the disease and further periodontal attachment loss by providing an environment to reduce the accumulation of microbial pathogens. Antibiotics are used as an adjunct to control the periodontal disease activity. All the antibiotics used in periodontal therapy work to eliminate the major periodontal pathogens such as *P. gingivalis*, *C. rectus* and *Capnocytophaga*. Minocycline is the most effective antibiotic used in inhibition of the growth of periodontal pathogens (Anoop et al., 2012).

Results of the study by Griffiths et al. and Kaner et al. reported that administration of combinations of systemic antibiotics such as metronidazole and amoxicillin (MTZ+ AMX) and (SRP+MTZ+AMX) immediately or 3 months after the mechanical debridement
varies (Kaner, et al., 2007). According to the study published by Griffiths, et al., systemic antibiotics administered during active phase of the periodontal therapy is more effective when compared to the administration of antibiotics during the healing phase (Griffiths, et al., 2011). This is possibly due to the new stable biofilm community formed by the rapid and striking decrease of the microbial pathogens in recently scaled pockets. This stable biofilm niche is similar to the one seen in healthy individuals (Karin & Søren, 2016).

In a local delivery method smaller doses of topical agents are delivered inside the pocket. The use of the local delivery method alone for administering antibiotics is an unconventional method of treatment for localized infections as this method does not provide superior results. Local delivery of antibiotics in combination with scaling and root planning (SRP) is most effective in sites that do not respond to conventional methods. Scaling and local delivery of antibiotics is a treatment approach in case of isolated areas with recurrent disease while, scaling with systemic antibiotics is an approach to treat generalized recurrence of the disease (Abinaya et al., 2012).

There is limited information on the adverse events following antibiotic therapy in the literature. Most of the side effects reported are minor and are related to gastrointestinal problems. However, serious adverse events such as anaphylactic and allergic reactions were reported with the administration of penicillin and 10% of the side effects were considered fatal (Wilson et al., 2007). Microbial resistance following antibiotics therapy was reported in few studies (Feres, et al., 2002). Systemic antibiotics are useful
antimicrobial agents for the management of periodontal disease when used in combination with mechanical debridement for the disruption of the subgingival biofilm.

**Role of antioxidants in treatment of periodontitis:**

Periodontal disease increases the number of polymorphonuclear leukocytes (PMN) present and its activity. Localized PMNs cause oxidative damage to the gingival tissue, periodontal ligament and alveolar bone. The damage caused by the free radicals can be reduced by the antioxidant defense system (Gowri, Biju, & Suchetha, 2008).

Though antibiotics can restrain the micro-organisms that contribute to periodontitis, they cannot directly act on the free radicals and oxidative stress that support inflammation. Cells require adequate amounts of antioxidants in order to prevent damage to the tissue by the free oxygen radicles (Pooja, 2016). Nitric oxide and oxidative stress play an important role in pathogenesis of periodontitis. Apart from these, hydrogen peroxide which is known to produce toxic gases in subgingival pocket, is considered linked to oxidative stress and periodontal disease. H2S producing cells in the oral cavity are available in the human periodontal ligament stem cells. Cystathionine synthase and lyase are the main source of endogenous hydrogen sulfide (H2S). H2S plays an important role in bacteria-induced inflammatory response due to its pro-inflammatory properties. H2S is said to have both antioxidant properties by becoming cytoprotective and also to cause oxidative stress and is cytotoxic (Maria, et al., 2016).

The human body has natural antioxidants in saliva that can fight the free radicals by neutralizing them reducing inflammation. Antioxidants are available from various
sources including vitamins, minerals, hormones, enzymes and herbal supplements (Ravneet Kaur, et al., 2016). Several scientific articles revealed that many topical antioxidants are available which can supplement the natural antioxidants (Edward A., 2012) (Samuel B., 2012). Additionally, antioxidants have been found to reduce the severity of gingivitis, bleeding on probing and pocket depth. Hence, topical application of antioxidants may provide additional benefits to people with periodontal disease by reducing inflammation and also this is the most convenient method used in the dental settings (Hans et al., 2012).
SPECIFIC AIMS:

The present interventional study aims to assess the efficacy of a newly formulated dental foam in reducing gingival inflammation. The hypothesis is that brushing with the new foam possessing antioxidant properties plus brushing with a standard toothpaste, is superior to brushing with a standard toothpaste alone in reducing gingival inflammation in gingivitis and/or periodontitis. This project was primarily designed to test this hypothesis using the data sets of clinical periodontal measures including gingival index (GI), bleeding on probing (BOP), pocket depth, clinical attachment level and amount of plaque. The primary endpoint measured was the change from baseline in gingival index and bleeding on probing at Day 42 in test and control groups. Secondary outcomes included change in plaque amount, and pocket depth from baseline to day 14, 28 and 42 compared to the control group and also gingival index, BOP from baseline to day 14 and day 28. This study was conducted in 36 subjects with gingivitis and/or moderate periodontitis and aimed at decreasing the gingival inflammation by administration of new dental foam over a period of 42 days to observe the earliest changes in gingival inflammation.

This study should help determine the efficacy of the newly formulated foam in reducing periodontal disease progression.
METHODS:

The data sets used in this analysis are obtained from a randomized clinical pilot trial conducted at the Center for Clinical and Translational Research at the Forsyth Institute, Cambridge MA. The characteristics of the subject population included being medically healthy, 18-60 years of age, clinically diagnosed with gingivitis and/or periodontitis with a minimum of 20 natural teeth excluding third molars.

General exclusion criteria included 1) chronic use of medications including non-steroidal anti-inflammatory drug (NSAID’s), antidepressants, antibiotics, and beta blockers, 2) diabetes 3) participating in another trial or the use of other oral study products, 4) pregnant or breast feeding women, 5) use of antibiotic within three months, 6) history of drug use that is associated with gum overgrowth (Dilantin, Nifedipine), 7) chronic use of anti-coagulants, immunosuppressors, steroids, 8) medical condition which requires pre-medication prior to dental visits/procedures, and 9) current smokers and former smokers within one year of enrollment. Dental exclusion criteria included 1) presence of orthodontic appliances, 2) soft or hard tissue tumor of oral cavity, 3) carious lesions that require immediate treatment, and 4) patients with severe chronic periodontitis, aggressive periodontitis, acute necrotizing ulcerative gingivitis or generalized gingival recession.

The study protocol was approved by the Forsyth Institutional Review Board (FIRB). A total of 36 subjects were enrolled after a thorough screening process based on the inclusion/exclusion criteria followed by the informed consent process (Forsyth IRB #15-05). This study was a single blinded study, where the examiner was blinded to the group assignment. In randomized controlled trials, blinding is performed to reduce
intentional or unconscious bias by assessors and/or participants (Paul et al., 2010). Eligible subjects were scheduled for a baseline visit. The subjects were randomly assigned to treatment groups using a block size of six in a randomization schedule using a publicly available website. The website is randomization.com (http://www.tufts.edu/~gdallal/randomcite.htm). Randomization.com uses the pseudorandom number generator of Wichmann and Hill (1982) as modified by McLeod (1985) (Suresh, 2011). Subjects were randomized using a 2:1 randomization scheme into either 1) brushing with test product + standard toothpaste or 2) brushing with standard toothpaste alone. The formulation of the test product used in this study is a propriety formulation. The main ingredients of the test product, antioxidants, are reportedly known to reduce gingival inflammation (Graziani, et al., 2015). Also, this is a new approach with new formulations using antioxidants similar to toothpaste and is tested on existing gingivitis and periodontal inflammation. Subjects in the control group were instructed to brush their teeth with standard tooth paste alone twice a day for two minutes and those in the test group to brush with the test product together with the toothpaste twice a day for 2 minutes. Subjects were asked to return to the clinic at day 5, 14, 28 and 42 for safety and periodontal evaluations. Subjects were not permitted to use other dental hygiene products during the course of the study.

Clinical periodontal measurements including pocket depth, gingival index, bleeding on probing and plaque index were recorded at baseline (Day 0) and repeated at each monitoring visit on Day 14, 28, and 42 following baseline. Safety evaluations
including adverse events and oral soft and hard tissue evaluation were recorded at baseline and repeated on Day 5, 14, 28, and 42.

**Periodontal clinical assessments:**

Gingival index (GI) (Löe and Silness, 1963) and plaque index (PI) (Silness and Löe, 1964) (Poulsen, 1981) were used to measure the degree and the extent of the gingival inflammation and the quantity of plaque. For the gingival index, the UNC-12 periodontal probe was placed slightly under the gingival margin and gently swept along the buccal and lingual surfaces to detect bleeding tendency and measure the severity of the inflammation. For the assessment of plaque, the probe is swept along the gingival margin on tooth surfaces (buccal and lingual surfaces) and the visible plaque is noted (Table 1).

Probing depth, a measurement of the depth of the sulcus, was evaluated by measuring the distance from the gingival margin to the base of the sulcus with a UNC-12 periodontal probe (Table 1, Figure 5). Six sites per tooth were measured and rounded down to the next whole mm (Figure 6). The clinical attachment level is the measurement of position of soft tissue with regard to the cemento-enamel junction (CEJ), which is a fixed point that does not change. The two measurements used to determine the clinical attachment level (CAL) are the probing depth and the distance from gingival margin to the CEJ (Fritz, 2013)(Figure 5). Bleeding on probing was assessed after periodontal probing. This is a dichotomous scoring system which is used at six sites per tooth using one (1) and zero (0) for the presence or absence of bleeding 15 seconds after probing.
Table 1 Scores for Bleeding on probing, Gingival Index and Plaque Index

Standard criteria used to assess gingival health using scores from 0 to 3. Abbreviations BOP: bleeding on probing. Table amended from Carranza’s Clinical Periodontology, 1984.

<table>
<thead>
<tr>
<th>Scores</th>
<th>Bleeding on Probing</th>
<th>Gingival Index</th>
<th>Plaque Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Absence of bleeding</td>
<td>Normal gingiva</td>
<td>No plaque</td>
</tr>
<tr>
<td>1</td>
<td>Presence of bleeding</td>
<td>Mild inflammation change in color, slight edema, No BOP</td>
<td>Film at gingival margin</td>
</tr>
<tr>
<td>2</td>
<td>Presence of bleeding</td>
<td>Moderate inflammation: redness, edema, BOP</td>
<td>Moderate (easily visible)</td>
</tr>
<tr>
<td>3</td>
<td>Presence of bleeding</td>
<td>Severe inflammation: marked redness &amp; edema, ulceration and spontaneous bleeding</td>
<td>Abundance of material</td>
</tr>
</tbody>
</table>
Figure 5 Probing depth;
Entire sulcus is probed using UNC-12 and the distance between the Cemento-enamel junction and gingival margin is recorded and is rounded to nearest mm (Preshaw, et al., 2012)
Figure 6 Anatomy of a molar
Shown are the six sites per tooth that are assessed, 3 buccal and 3 lingual sites (Edward, 2007)

Statistical Analysis:

All statistical analysis was conducted using Microsoft excel. In an Intent-to-Treat (ITT) analysis, the data collected on all randomized subjects at any post-baseline visit was included in the data analysis despite any missing visits. The data was presented as mean ± standard deviation (SD). The main objective of the study was to assess the change in gingival index and BOP from baseline to day 42 compared to the control group. This clinical data is measured by taking an average mean of all the indices for each subject and then the change from baseline to each point is calculated for each person. These changes would then be compared between the test group and the control group. Secondary measures
included the change in gingival index, BOP, plaque amount, and pocket depth from baseline to day 14, 28, and 42 compared to the control group.

The treatment changes from baseline to each time point were evaluated using the paired t-test. A level of $p < 0.05$ was considered statistically significant. Differences between baseline and day 14, 28 and 42 between test and control groups were compared using unpaired t-test. The categorical variables between groups including gender and race were analyzed using a chi-square test.
RESULTS

The clinical data from a total of 36 subjects (19 females (53%) and 17 males (47%)) participating in the study were included in this analysis testing the efficacy of a newly formulated dental product in reducing gingival inflammation compared to a control group.

Table 2 represents the demographic characteristics and Table 3 represents the clinical characteristics of both study groups. Descriptive statistics and the mean values of the clinical parameters are shown in Table 5. The study sample constituted of 53% females and 47% males, and were primarily (45%) white. The second largest racial category were black who constituted 40% of the study sample and the remaining subjects were comprised of Asians, Hispanic and mixed race representing the racial distribution of Greater Boston and Cambridge areas. The mean age of the study sample was 42 years (+/-11.44 SD) (Table 2).
Table 2 Demographic characteristics of study participants by group
Descriptive statistics for demographics in both groups

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Test group n=24</th>
<th>Control group n=12</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean ± SD)</td>
<td>41.17 ± 11.44</td>
<td>39.08 ± 12.61</td>
<td>0.64</td>
</tr>
<tr>
<td>Gender, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>10 (41.6%)</td>
<td>7 (58.3%)</td>
<td>0.35</td>
</tr>
<tr>
<td>Female</td>
<td>14 (58.3%)</td>
<td>5 (41.6%)</td>
<td></td>
</tr>
<tr>
<td>Race, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixed</td>
<td>2 (8.3%)</td>
<td>1 (8.3%)</td>
<td>0.17</td>
</tr>
<tr>
<td>Asian</td>
<td>3 (12.5%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>12 (50%)</td>
<td>4 (33.3%)</td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>5 (20.8%)</td>
<td>7 (58.3%)</td>
<td></td>
</tr>
<tr>
<td>Hispanic</td>
<td>2 (8.3%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
</tbody>
</table>

Table 3 Baseline clinical parameters of study participants
Gingival index, bleeding on probing, plaque index and pocket depth were measured in control subjects as well as test subjects treated with the newly formulated product. Data presented as Mean± standard deviation (s.d.).

<table>
<thead>
<tr>
<th>Clinical parameters</th>
<th>Test group n=24</th>
<th>Control group n=12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gingival Index</td>
<td>1.86 ± 0.19</td>
<td>1.84 ±0.12</td>
</tr>
<tr>
<td>Bleeding on Probing (%)</td>
<td>35%</td>
<td>35%</td>
</tr>
<tr>
<td>Plaque Index</td>
<td>1.30 ± 0.34</td>
<td>1.15 ±0.46</td>
</tr>
<tr>
<td>Pocket Depth</td>
<td>2.19 ± 0.30</td>
<td>2.33 ±0.33</td>
</tr>
</tbody>
</table>
Table 4 Change in Gingival Index and Bleeding on Probing from baseline to day 42:

Element of primary outcome - comparison of gingival index (GI) and bleeding on probing (BOP) change in control and test groups from baseline to day 42. Data presented as mean ± standard deviation.

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline GI</th>
<th>Day 42 Gingival GI</th>
<th>Baseline BOP</th>
<th>Day 42 BOP</th>
<th>Change= Day 42- baseline</th>
</tr>
</thead>
</table>
| Test     | 1.86 ± 0.19 | 1.63 ± 0.19       | 35%          | 26%        | GI= -0.23
            |             |                    |              |            | BOP = -8%                  |
| Control  | 1.84 ±0.12 | 1.83 ± 0.16       | 35%          | 36%        | GI= -0.01
            |             |                    |              |            | BOP = 1%                   |

According to table 4 and table 5, we can see that there is a very minimal change between the test and the control group at baseline, which dramatically changed at day 42.
Figure 7 Change in Gingival Index from baseline to Day 42. Shown is the change in gingival index (GI) between control and test subjects following use of the newly formulated product. Data collected a baseline and at day 42.
Figure 8 Change in Bleeding on Probing from baseline to day 42
Shown is the change in bleeding on probing (BOP) between control and test subjects following use of the newly formulated product. Data collected at baseline and at day 42.

The change in baseline to day 42 for GI and BOP are the primary outcome measures. There was a statistically significant reduction in gingival index and bleeding on probing from baseline to day 42 in the test group compared to control group (p<0.003 and p<0.007, respectively) (Table 5) and Figure 7, Figure 8.

Table 5. Differences in Mean Gingival Index, Bleeding on Probing, Plaque Index and Probing Depth at baseline, Day 14, Day 28 and Day 42 between groups
Shown is the change in gingival index (GI) between control and test subjects following use of the newly formulated product. Time points collected include baseline and day 14, 28 and 42. Data presented as mean ± standard deviation. Statistically significant difference compared to control group (p<0.05) shown by asterisk*.

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Time point</th>
<th>Test group (n=24)</th>
<th>Control group (n=12)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gingival Index</td>
<td>Baseline</td>
<td>1.86 ± 0.19</td>
<td>1.84 ± 0.12</td>
<td>0.62</td>
</tr>
<tr>
<td></td>
<td>Day 14</td>
<td>1.71 ± 0.2</td>
<td>1.76 ± 0.16</td>
<td>0.44</td>
</tr>
<tr>
<td></td>
<td>Day 28</td>
<td>1.67 ± 0.19</td>
<td>1.79 ± 0.14</td>
<td>0.05*</td>
</tr>
<tr>
<td></td>
<td>Day 42</td>
<td>1.63 ± 0.2</td>
<td>1.83 ± 0.16</td>
<td>0.003*</td>
</tr>
<tr>
<td>Bleeding on Probing</td>
<td>Baseline</td>
<td>35%</td>
<td>35%</td>
<td>0.91</td>
</tr>
<tr>
<td></td>
<td>Day 14</td>
<td>28%</td>
<td>31%</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>Day 28</td>
<td>28%</td>
<td>35%</td>
<td>0.05*</td>
</tr>
<tr>
<td></td>
<td>Day 42</td>
<td>26%</td>
<td>36%</td>
<td>0.007*</td>
</tr>
<tr>
<td>Plaque Scores</td>
<td>baseline</td>
<td>1.30 ± 0.34</td>
<td>1.15 ± 0.46</td>
<td>0.35</td>
</tr>
<tr>
<td></td>
<td>Day 14</td>
<td>1.06 ± 0.35</td>
<td>1.23 ± 0.43</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>Day 28</td>
<td>1.05 ± 0.37</td>
<td>1.21 ± 0.51</td>
<td>0.35</td>
</tr>
<tr>
<td></td>
<td>Day 42</td>
<td>0.93 ± 0.36</td>
<td>1.18 ± 0.38</td>
<td>0.07</td>
</tr>
<tr>
<td>Probing Depth</td>
<td>baseline</td>
<td>2.19 ± 0.3</td>
<td>2.33 ± 0.33</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>Day 14</td>
<td>2.13 ± 0.3</td>
<td>2.31 ± 0.34</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>Day 28</td>
<td>2.14 ± 0.31</td>
<td>2.30 ± 0.32</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td>Day 42</td>
<td>2.13 ± 0.31</td>
<td>2.33 ± 0.37</td>
<td>0.12</td>
</tr>
</tbody>
</table>
A statistically significant difference was found between GI (p <0.003) and BOP (p<0.007) in the test group from baseline to day 42 when compared with the control group. Apart from being statistically significant, these results are clinically meaningful. The changes that are seen in the intervention group are clinically important. The mean values for probing depth and plaque index also showed a reduction from baseline to day 42 in test group, however, when compared to control group, the differences were not statistically significant (p<0.12 and p < 0.07, respectively) (Table 6).

![Figure 9 Change in Gingival Index from baseline to day14, day 28 and day 42](image)

Shown is the change in Gingival Index(GI) between control and test subjects following use of the newly formulated product. Data collected at baseline and at day 14, 28, 42.
Figure 10 Change in Bleeding on Probing (BOP) from baseline to day 14, day 28, day 42. Shown is the change in Bleeding on Probing (BOP) between control and test subjects following use of the newly formulated product. Data collected at baseline and at day 14, 28, 42.

Secondary outcome measures include the reduction in plaque index, and pocket depth from baseline to day 14, 28 and 42 and change in gingival index and BOP from baseline to days 14 and 28. Change in plaque scores decreased from baseline (0.24) to day 42 in test group (0.12), while in control group the plaque scores increased at day 14 and decreased at day 42 (Table 7) (Figure 11). Changes in pocket depth were not found to be statistically different. The probing pocket depth showed incremental but steady change over time in the test group. Figure 12 and Table 7.
Figure 11 Change in Plaque Index (PI) from baseline to day 14, day 28 and day 42. Shown is the change in Plaque Index (PI) between control and test subjects following use of the newly formulated product. Data collected at baseline and at day 14, 28, 42.
Figure 12 Change in Pocket Depth (PD) from baseline to day 14, day 28 and day 42. Shown is the change in Pocket Depth (PD) between control and test subjects following use of the newly formulated product. Data collected at baseline and at day 14, 28, 42.
Table 6 Change in Clinical parameters from Baseline to Day 42

Comparison of mean change between the intervention and control group at each time point starting at baseline to day 14, day 28 and to day 42. ↓ Decrease in change between time points, ↑ Increase in change between time points.

<table>
<thead>
<tr>
<th></th>
<th>Change in the clinical parameters from baseline to day 42</th>
<th>Intervention group (n=24)</th>
<th>Control group (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gingival Index</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline to Day 14</td>
<td>0.15 ↓</td>
<td>0.08 ↓</td>
<td></td>
</tr>
<tr>
<td>Day 14 to Day 28</td>
<td>0.04 ↓</td>
<td>-0.03 ↑</td>
<td></td>
</tr>
<tr>
<td>Day 28 to Day 42</td>
<td>0.04 ↓</td>
<td>-0.04 ↑</td>
<td></td>
</tr>
<tr>
<td><strong>Bleeding on Probing</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline to Day 14</td>
<td>7% ↓</td>
<td>(No change)</td>
<td></td>
</tr>
<tr>
<td>Day 14 to Day 28</td>
<td>(No change)</td>
<td>4% ↑</td>
<td></td>
</tr>
<tr>
<td>Day 28 to Day 42</td>
<td>2% ↓</td>
<td>1% ↑</td>
<td></td>
</tr>
<tr>
<td><strong>Plaque Scores</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline to Day 14</td>
<td>0.24 ↓</td>
<td>-0.08 ↑</td>
<td></td>
</tr>
<tr>
<td>Day 14 to Day 28</td>
<td>0.01 ↓</td>
<td>0.02 ↓</td>
<td></td>
</tr>
<tr>
<td>Day 28 to Day 42</td>
<td>0.12 ↓</td>
<td>0.03 ↓</td>
<td></td>
</tr>
<tr>
<td><strong>Probing Depth</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline to Day 14</td>
<td>0.06 ↓</td>
<td>0.02 ↓</td>
<td></td>
</tr>
<tr>
<td>Day 14 to Day 28</td>
<td>-0.01 ↑</td>
<td>0.01 ↓</td>
<td></td>
</tr>
<tr>
<td>Day 28 to Day 42</td>
<td>0.01 ↓</td>
<td>-0.03 ↑</td>
<td></td>
</tr>
</tbody>
</table>
Changes within the group have been evaluated using the paired t-test. The change within the group between each time point is calculated and is compared with the other group (Table 7). There was a significant reduction in gingival index from baseline (0.15) to day 42 (0.04) in the test group. Conversely, in the control group, the mean gingival index increased from baseline (0.08) to day 42 (-0.04). Likewise, bleeding on probing dramatically reduced at day 42 compared to baseline (35% to 26%) in the test group. The changes in control group, however, were smaller and were not significantly different (Table 7 and Figure 10). The descriptive analysis and statistical tests compared the data between groups revealed that the mean values of gingival inflammation and bleeding on probing significantly reduced in the test group when compared to the control group at both day 28 and 42).
DISCUSSION

In this randomized clinical study, we analyzed the efficacy of a new dental product on the reduction of gingival inflammation as measured by the periodontal clinical indices GI, BOP, PI, and PD. Subjects were assigned to either the test group brushing with the new product along with a standard toothpaste or to the control group brushing with a standard toothpaste only. Data collected demonstrated that the newly formulated dental product, when used in conjunction with a commercially available toothpaste, resulted in significant reduction in gingival inflammation over a 42-day period in subjects with gingivitis or periodontitis. The control group receiving toothpaste alone showed increased level of gingival inflammation over the same period. The study results indicate that the newly formulated dental product is effective in controlling gingival inflammation and maintaining gingival health in subjects susceptible to gingival inflammation and gingival diseases.

There was a significant reduction in GI and BOP values in the test group receiving the new foam. This could be attributed to the antioxidant properties of the product. The gingival bleeding is an important indicator of risk for periodontitis (Lucarini, et al., 2008). Gingival bleeding helps in assessing the clinical attachment level changes and hence serves as a surrogate measure in the prevention of periodontitis. There was a steady decrease in mean bleeding on probing percentage from 35% at the baseline to 26% at day 42 which further indicates the control of inflammation and oral health status.

With respect to the findings from the review by Needleman et.al, there is greater reduction in gingival bleeding and plaque when treated with professional mechanical plaque removal and oral hygiene instructions (OHI) when compared to no treatment
(Needleman et al., 2015). Also, there is some evidence from these studies that the plaque, bleeding and attachment loss benefit more from frequent mechanical plaque removal. The decrease of plaque index in the current study is not statistically different but there was a noticeable amount of reduction in plaque score in test group (1.30-0.93 points) assigned to new product when compared to the control group (1.15-1.18 points). Some studies have shown a significant reduction in pocket depth in both deeper and shallow sites following a skilled tooth brushing technique with follow up (Takashi et al., 2004). Conversely, in our study even though the reduction in pocket depth was not statistically significant, there was a reduction in pocket depth in test group (2.19-2.13 points), when compared to the control group (2.33-no change).

There was a significant reduction in bleeding on probing (p<0.007) in the test group that used the new foam together with toothpaste when compared to the control group that used the toothpaste alone. This is in accordance to the study by Gonzalez et al. in which bleeding on probing decreases substantially after professional mechanical removal of plaque but there was not much improvement after conventional oral hygiene alone (Gonzalez, et al., 2014).

A report by European Workshop of Periodontology supported the universal recommendations to brush twice daily for two minutes (Chapple, et al., 2015). Whereas, in a meta-analysis that reviewed much of clinical trials between 2002 and 2005 found that mechanical removal of plaque using tooth brush combined with removal of interdental plaque once in 24 hours is adequate to prevent the onset of gingival inflammation (Akshay & Vandana, 2012). In this context our results provide clinical evidence to maintain gingival
health with the use of a newly formulated dental foam along with standard tooth brushing twice daily for two minutes. Strengths of the study include the study design and assessment of compliance. Randomization helps in providing the comparable groups at baseline and removes any confounding variables. Blinding reduces the measurement bias that could be caused by the examiner. Study limitations include stringent inclusion criteria which question the validity of the study in large subject populations due to the difficulty in generalizing the results, as the studied population is different from the population in normal life. Thus, the results of this foam might differ in the population outside the study. Another limitation of the study would be that subjects were restricted from using other dental aids which in reality is difficult to apply. One future studies could be a longitudinal clinical trial including large and diverse population with severe periodontal disease.

**CONCLUSIONS**

In this study, reduced scores of gingival index (GI) and bleeding on probing (BOP) were observed in the test group using newly developed foam along with standard toothbrush and paste compared to a control group using tooth brush and paste alone. These results suggest that the new dental product with antioxidant properties present efficacious changes in gingival inflammation which could be beneficial to the daily oral hygiene of subjects susceptible to gingivitis and periodontitis.
## LIST OF JOURNAL ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Title</th>
</tr>
</thead>
<tbody>
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