

# Anti-gingivitis effect of a dentifrice containing bioactive glass (NovaMin<sup>®</sup>) particulate

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Tai BJ, Bian Z, Jiang H, Greenspan DC, Zhong J, Clark AE, Du MQ. Anti-gingivitis effect of a dentifrice containing bioactive glass (NovaMin<sup>®</sup>) particulate. *J Clin Periodontol* 2006; 33: 86–91. doi: 10.1111/j.1600-051X.2005.00876.x. © Blackwell Munksgaard 2006.

## Abstract

**Background:** The objective of this pilot clinical trial was to evaluate the anti-gingivitis and anti-plaque effects of a dentifrice containing bioactive glass (NovaMin<sup>®</sup>) compared with a placebo control dentifrice in a 6 weeks clinical study.

**Methods:** The study design was a randomized, double-blinded, controlled clinical trial. One hundred volunteers took part in the study and were matched for plaque index (PLI), gingival bleeding index (GBI), age and gender. The protocol was reviewed and approved by the Ethical Committee of the University. The subjects received a supragingival prophylaxis to remove all plaque, calculus and extrinsic stain. Following the baseline examination, subjects were instructed to brush with their assigned dentifrice and toothbrush. The PLI and GBI were determined for the baseline and 6 weeks. The data were analysed using a repeated-measures ANOVA conducted on the two dependent measures to compare the effect between the test and control group.

**Results:** Ninety-five subjects finished the study. The results showed that the PLI (baseline = 1.54, 6 weeks = 1.29) and GBI (baseline = 1.14, 6 weeks = 0.47) were significantly reduced, respectively, over the 6 weeks period in the test group ( $p < 0.001$  for each measure). There was a 58.8% reduction in gingival bleeding and a 16.4% reduction in plaque growth. There was no difference of the PLI (baseline = 1.60, 6 weeks = 1.57) and GBI (baseline = 1.18, 6-week = 1.02) over the 6 week period in the control group.

**Conclusion:** This study demonstrated that a dentifrice containing NovaMin<sup>®</sup> significantly improves oral health as measured by a reduction in gingival bleeding and reduction in supragingival plaque compared with a negative dentifrice over the 6 weeks study period.

Key words: anti-gingivitis, anti-plaque, bioactive glass.

Accepted for publication 24 October 2005

Maintenance of gingival health is critical in preventing gingivitis and its progression into periodontal disease. A recent survey of periodontal health reported that as many as 62% of the adult population in the United States suffers from gingivitis as determined by gingival bleeding (Brown et al. 1996). A similar study conducted in the United Kingdom reported that over 70% of adults had visible plaque on examination (Morris et al. 2002).

The control of plaque in the maintenance of gingival health has been well

established in the literature (Axelsson & Lindhe 1981, Cobb 1996). The role of plaque-associated bacteria in the development of gingivitis has also been studied extensively (Smulow et al. 1983, Dahlen et al. 1992, Marsh 1992, McNabb et al. 1992). Smulow et al. (1983) demonstrated that professional plaque control had a significant influence on subgingival bacterial levels. McNabb et al. (1992) and Hellstrom et al. (1996) showed that rigorous self-performed plaque control over long periods of time reduced the levels and

composition of subgingival bacteria and reduced the frequency of deep periodontal pockets.

Owing to the established relationship between bacteria, plaque and gingivitis, a major focus in the treatment of gingivitis in recent years has been the development and use of various antimicrobial therapies. Chlorhexidine (Clavero et al. 2003, Santos et al. 2004), triclosan/co-polymer (Rosling et al. 1997, Nogueira-Filho et al. 2000, Volpe et al. 2002, Cullinan et al. 2003) and hexetidine (Sharma et al. 2003) have all

been shown to reduce plaque and gingivitis in various clinical studies. Essential oil-containing mouthrinses have also demonstrated reductions in plaque and gingivitis in clinical studies (Lush et al. 1974, Charles et al. 2004, Sharma et al. 2004). Many of these clinical studies have demonstrated improvements in indices of gingival health, which have been ascribed to the anti-microbial properties of the various compounds. Rosling et al. (1997) showed an effect of a triclosan-containing dentifrice on the number of subgingival microbiota over a 3-year period, although these effects were limited to only a few of the species evaluated. In a short-term clinical study, Adams et al. (2003) showed a significant reduction in the viability of plaque bacteria in a triclosan/zinc formulation compared with a triclosan/polymer toothpaste containing no zinc. In a review of clinical data for two chemotherapeutic mouthwashes, Santos (2003) concluded that studies demonstrated a clinical benefit in reducing plaque and gingivitis and resulted in a lowering of the total microbial flora without changing the microbial composition of supragingival plaque.

There has also been a significant body of *in vitro* research that has demonstrated anti-microbial properties of these compounds. In a study comparing the anti-microbial efficacy of a triclosan/zinc dentifrice, Finney et al. (2003) found a broad spectrum of anti-microbial activity against a mixed biofilm. Shapiro et al. (2002) demonstrated a significant reduction in microbial load for 12 different mouthrinse products in a mixed biofilm model.

Bioactive glasses (NovaMin<sup>®</sup>, NovaMin Technology, Alachua, FL, USA) have been used in bone and tissue regeneration for over 15 years (Wilson et al. 1993, Lovelace et al. 1998). Recently, anti-microbial properties inherent in these materials have been described (Stoor et al. 1998, Allan et al. 2001, 2002). One of these compositions has recently been formulated into a dentifrice and has demonstrated strong anti-microbial behaviour *in vitro* (Greenspan et al. 2004). While the exact mechanisms of the anti-microbial activity have not yet been fully established, it is likely that the high rate of ionic release and local changes in oral pH seem to play a major role.

This double-blind, placebo-controlled, pilot clinical study was designed to test the hypothesis that the anti-microbial effects of NovaMin<sup>®</sup> shown

*in vitro* when incorporated into a daily use dentifrice could improve the indices of gingival health in a general population with no periodontitis.

### Material and Methods

The study was a randomized, double-blind, parallel group clinical trial comparing experimental dentifrice with an active NovaMin<sup>®</sup> to a placebo formulation. The protocol for the study was reviewed and approved by the Medical Ethical Committee of Wuhan University. All volunteers provided written informed consent for participation in the study. The study was conducted and monitored in accordance with the guidelines for Good Clinical Practice. Two hundred volunteers from the local population were recruited for the study through local advertising and clinical patients from the clinics at Wuhan University. One hundred qualified volunteers were accepted into the study.

The inclusion criteria required patients to be at least 18 years of age, in good general health, with a minimum of 20 scorable teeth, no visible signs of untreated caries and in good general health. Exclusion criteria included patients who received antibiotics or anti-inflammatory therapy within 14 days of the baseline examination or were on long-term antibiotic or anti-inflammatory therapy, patients who had periodontal pockets in excess of 4 mm, no partial dentures or clinically unacceptable restorations or bridges, pregnant or lactating women and patients with removable dentures (partial or full).

Sample size calculations were based on detecting a difference of 0.3 in plaque score between the test group and the control group using a two-tailed significance level of 5% with a 90% power. The Silness and Loe plaque index (PLI) was used in this study (Silness & Loe 1964). An average plaque score was calculated for each patient by summing the individual plaque scores for each tooth and dividing by the total number of sites scored for each subject. From the individual scores, mean group PLI scores were calculated. Gingival bleeding index (GBI, Ainamo & Bay 1975) was calculated for all teeth of each subject by summing the individual GBI scores and dividing that sum by the number of sites graded for each subject. These measurements were conducted at baseline and at 6-week recall visits. A two by two

(treatment: test material, placebo) by two (time period: baseline, 6 weeks) repeated-measures ANOVA analysis was conducted on two dependent measures, PLI and GBI. Means and SDs from these analyses are reported. Statistical analysis of the data was performed using SPSS Version 10 (SPSS, Chicago, IL, USA).

A complete clinical oral assessment was carried out including measurement of PLI and GBI at baseline and after 6 weeks. The assessments included the mesial, buccal, distal and lingual surfaces for plaque after using a plaque-disclosing treatment (Chrom-O-Red disclosing solution, Germiphene, Brantford, ON, Canada) and mesio-buccal, buccal and disto-lingual surfaces for gingival bleeding, which were recorded 30 s after running a probe along the gingival margin. A single examiner performed all clinical measurements for the clinical study. There was no calibration of the examiner prior to the study. Screening prior to acceptance into the study was conducted for each subject. Complete medical and dental history was reviewed, and examination of the oral mucosa, teeth and periodontium was performed.

Patients accepted into the study returned for a baseline examination. Patients were told not to perform any oral hygiene (including chewing gum) for 8 h prior to the baseline and 6 weeks examination. Patients were assessed for plaque using the PLI and gingival inflammation using GBI, as well as for oral soft-tissue status. Following the assessments, all subjects received a supragingival prophylaxis and polishing to remove plaque, calculus and extrinsic stain.

After prophylaxis, patients were instructed on the proper brushing technique and were given either the test dentifrice (non-aqueous toothpaste containing 5% NovaMin<sup>®</sup>) or a placebo formulation (non-aqueous toothpaste without NovaMin<sup>®</sup>) along with a diary to record product usage and daily oral hygiene activities. The abrasivity of both dentifrices were similar: the relative dentin abrasivity (RDA) of the active was  $130 \pm 7.3$ , and the RDA of the placebo was  $114 \pm 5.8$  (data on file at NovaMin Technology Inc., Alachua, FL, USA). The test and control dentifrices were dispensed to subjects by a dental assistant not involved in the study. The two products were also similar in terms of their taste, texture and colour. All products were packaged in plain white tubes (50 g each) labelled

Table 1. Demographic data of the subjects

	Test group	Control group
<i>n</i>	47	48
Female	22	25
Male	25	23
Age (mean)	34.5	38.6
Standard deviation	8.8	10.1

Table 2. Effects on plaque index (PLI) at two time periods (means and standard deviations)

	Test group	Control group
Subjects	47	48
Baseline	1.54 (0.34)	1.60 (0.37)
6 weeks	1.29 (0.40)	1.57 (0.41)
Mean difference (baseline–6 weeks)	0.25 (0.08)	0.03 (0.09)
Baseline–6 weeks	$p < 0.001$	$p > 0.05$

only with lot numbers to insure proper blinding of the product from the patients and an examiner. Subjects were also given a soft bristled toothbrush to use during the clinical study. Subjects were assigned to one of the toothpastes using a computer-generated randomization numbering scheme. Subjects were asked to refrain from all other unassigned forms of oral hygiene, including non-study toothbrushes or toothpastes, dental floss, chewing gum or oral rinses during the study. At 6 weeks, subjects were recalled and gingival index and PLI were evaluated. At the conclusion of the study, all study materials were returned to the study director.

## Results

A total of 200 students were screened and 100 subjects were enrolled, with 50 subjects per product group. A total of 95 patients completed the study. All diaries to record product usage and daily oral hygiene activities were retrieved, and information violating rules of the study were not found. Five subjects did not complete the study because of personal or medical reasons unrelated to the use of study toothpastes. No adverse events were recorded or observed during the study. The demographic data for the subjects completing the study are shown in Table 1.

Table 2 shows the mean values and SDs of the PLI for all subjects who completed the study. The differences between the test group and the control group at baseline were not statistically significant. The PLI was reduced

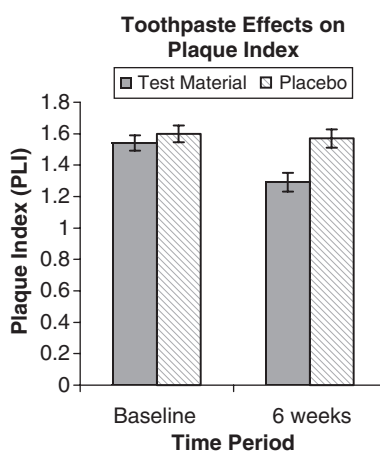


Fig. 1. Results of the plaque index (PLI) measurements at baseline and 6 weeks. There was no statistical difference between groups at baseline, and the test group had significantly lower PLI at 6 weeks compared with the control group ( $p < 0.001$ ). There was also a significant decrease in PLI of the test group at 6 weeks compared with baseline ( $p < 0.001$ ), while there was no significant decrease in PLI for the control group between baseline and 6 weeks. The error bars represent the standard error of the mean.

Table 3. Effects on gingival bleeding (GBI) at two time periods (means and standard deviations)

	Test group	Control group
Subjects	47	48
Baseline	1.14 (0.79)	1.18 (0.71)
6 weeks	0.47 (0.36)	1.02 (0.56)
Mean difference (baseline–6 weeks)	0.67 (0.15)	0.16 (0.15)
Baseline–6 weeks	$p < 0.001$	$p > 0.05$

significantly ( $p < 0.05$ ) in the test group after 6 weeks of product use. The reduction in the test group was 16.4%. There were no statistically significant differences in the PLI of the control group between baseline and 6-week measurements ( $p > 0.05$ ). At the 6-week time period, the reduction in PLI in the test group was significantly greater ( $p < 0.025$ ) compared with that in the control group (Fig. 1).

Table 3 shows the mean and SDs for the GBI data for all subjects who completed the study. The differences between the test group and the control group at baseline were not statistically significant. The GBI was significantly reduced in the test group compared with the baseline after 6 weeks of product use ( $p < 0.001$ ). The reduction in GBI in the test group after 6 weeks was

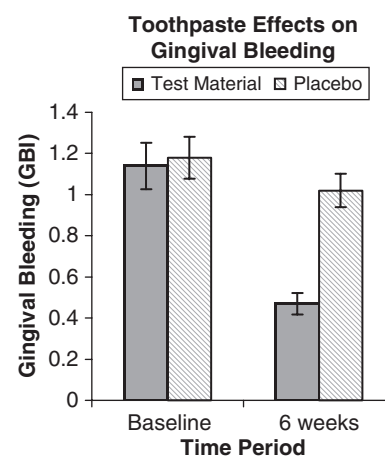


Fig. 2. Results of the gingival bleeding index (GBI) measurements at baseline and 6 weeks. There was no statistical difference between groups at baseline, and the test group had significantly lower GBI compared with the control group at 6 weeks ( $p < 0.001$ ). There was also a significant decrease in GBI of the test group at 6 weeks compared with baseline ( $p < 0.001$ ), while there was no significant decrease in GBI for the control group between baseline and 6 weeks. The error bars represent the standard error of the mean.

58.8%. There were no statistically significant differences between the baseline and 6-week data for the placebo toothpaste group ( $p > 0.05$ ). Comparison of the two groups at the 6-week time point showed significantly greater reduction in GBI for the test group compared with the control group ( $p < 0.001$ ) (Fig. 2).

## Discussion

The purpose of this pilot clinical study was to evaluate the effect of a new bioactive glass-containing dentifrice on the gingival health of an adult population with moderate gingivitis. The material was incorporated into a non-aqueous dentifrice formulation without fluoride and contained 5% by weight of the bioactive glass. The results demonstrated a significant reduction in GBI (58.8%) over a 6-week period of twice daily use, and a statistically significant reduction in PLI (16.4%) over that same period. In both cases, there was a statistically significant difference in both the PLI ( $p < 0.025$ ) and GBI ( $p < 0.001$ ) between the NovaMin<sup>®</sup> test group and the control group at the 6-week time point.

The lack of any appreciable reduction in either the PLI or GBI in the control group in this study was somewhat surprising. Many previous tooth-

brushing studies looking at gingival health and plaque reduction have shown significant treatment effects in the control groups (Owens et al. 1997, Heasman et al. 1999, McCracken et al. 2000). These effects have been attributed to the well-known Hawthorne effect. This phenomenon was first described by Mayo (1933) and is related to the fact that participants in a study will improve their performance regardless of any change in condition. In the current clinical study, it was expected that there would have been some improvement in the indices of the control group because of this effect, but it was not seen, even though all patients received the same toothbrushing instructions at the baseline examination.

While the mechanical control of dental plaque has been clearly shown to retard the advance of gingivitis and periodontal disease (Axelsson & Lindhe 1981, Wilson et al. 1987). Axelsson & Lindhe (1981) reported that non-compliant patients exhibited signs of recurrent disease processes. Owing to the inconsistency of simple mechanical control of plaque accumulation, a number of chemotherapeutic agents have been incorporated into home use products to control plaque and gingivitis. These agents have generally been incorporated into either mouthrinses or toothpastes. The main action of these agents has been focused on their anti-microbial action.

There have been a number of active ingredients incorporated into various dentifrices. Triclosan/co-polymer dentifrices with or without zinc citrate have been studied extensively for their anti-plaque and anti-gingivitis effectiveness. Triclosan is a phenolic agent comprised of bisphenol and a non-ionic germicide (DeVizio & Davies 2004). Lindhe et al. (1993) reported on the results of a 6-month clinical trial comparing a triclosan/co-polymer dentifrice with a fluoride-containing dentifrice and found that the triclosan group had more plaque reduction and resolution of gingivitis than the regular fluoride dentifrice group. Studies including long-term clinical trials (Rosling et al. 1997), short-term experimental gingivitis models (Nogueira-Filho et al. 2000) and short-term randomized clinical studies (Volpe et al. 2002) have demonstrated significant reductions in plaque and gingivitis from about 20% to as high as 60%.

Stannous fluoride has also been incorporated into various dentifrice compositions. Stannous fluoride is a broad spectrum anti-microbial agent. There

were a number of clinical studies conducted in the 1990s demonstrating the clinical efficacy of the material in the prevention and reduction of gingivitis. Perlich showed that a stabilized, 0.454% stannous fluoride dentifrice was superior to a NaF control in reducing gingival bleeding and gingival index, but not in plaque reduction of plaque (Perlich et al. 1995). In a 9-month comparative clinical study using both stannous fluoride dentifrices and mouthrinses in combination, Mengel et al. (1996) demonstrated statistically significant reductions in gingival and plaque indices for an amine/stannous fluoride regimen over NaF controls. In a clinical study comparing a stabilized stannous fluoride dentifrice with a triclosan/co-polymer dentifrice, McClanahan showed significant efficacy in the reduction of both gingivitis and gingival bleeding as well as statistically greater reductions in those parameters compared with the triclosan/co-polymer dentifrice (McClanahan et al. 1997). However, tooth staining and formulation challenges prevented wide acceptance of the material.

More recently, Archila et al. (2004) compared a stabilized stannous fluoride/sodium hexametaphosphate dentifrice with a triclosan-containing dentifrice as a control and found that the experimental group had statistically significantly less gingivitis and less bleeding than the control group. Mankodi et al. (2005) published work on a 6-month clinical trial with a stabilized 0.454% stannous fluoride/sodium hexametaphosphate dentifrice and showed a 21.7% reduction in gingivitis, 57.1% reduction in GBIs and 6.9% less plaque *versus* a fluoride dentifrice.

Early clinical studies using 0.2% chlorhexidine mouthrinses demonstrated an anti-plaque/anti-gingivitis effectiveness of chlorhexidine as an adjunct to normal daily oral hygiene (Loe et al. 1976, Lang et al. 1982). However, undesirable side effects including tooth staining and alteration of taste perception led to the use of lower concentrations of the anti-microbial agent. Charles et al. (2004) and others have demonstrated in clinical studies that reducing the concentration of chlorhexidine to 0.12% while effective at reducing plaque and gingivitis still resulted in tooth staining and calculus deposition in the group using the chlorhexidine mouthrinse.

Essential oil mouthrinses have also been shown to possess some effective-

ness against plaque and gingivitis. A study comparing a 0.12% chlorhexidine mouthrinse (Peridex<sup>®</sup>, Zila Technical, Phoenix, AZ, USA) with an essential oil mouthrinse (Listerine<sup>®</sup>, Pfizer, New York, NY, USA) showed comparable reductions in both plaque (21.6% and 18.8%, respectively) and gingival index (18% and 14%, respectively). Sharma et al. (2004) showed that an essential oil mouthrinse in conjunction with regular brushing and flossing provided a significant reduction in plaque and gingivitis over brushing and flossing alone.

Although bioactive glasses have been used in bone regenerative surgeries for over 15 years, it is only recently that the inherent anti-microbial properties of these materials have been studied. Stoor et al. (1998) first published results of exposure of planktonic organisms known to be involved in the progression of gingivitis and periodontitis to particulate bioactive glass compositions. In these studies, it was found that exposure of a 40% solution of bioactive glass to the cultures for 10 min. resulted in a 3 log reduction in *Actinobacillus actinomycetemcomitans* and total loss of viability of the organism with a 60 min. exposure. Similar results were found in *Porphyromonas gingivalis*. *Actinomyces naeslundii* lost total viability in a 10 min. exposure to the bioactive glass as did *Streptococcus mutans*. Allan et al. (2001), using particulates of bioactive glass (designated 45S5 Bioglass<sup>®</sup>, USBiomaterials Corp., Alachua, FL, USA) with a size range from 300 to 500 µm, showed an antibacterial effect against *S. mutans*, *S. sanguis*, *P. gingivalis*, *Furobacterium nucleatum*, *A. actinomycetemcomitans* and *Prevotella intermedia* with a 1-h exposure to the particulates. The percentage kill ranged from 51% against *S. mutans* to 100% kill of *P. intermedia*. Allan ascribed much of the effect of the bacterial kill to an increase in pH of the bioactive glass in solution, although it appears that the release of large quantities of calcium from the bioactive glass might also play a role in the observed behaviour towards the microbes. Allan et al. (2001) also studied this composition against mixed species biofilms derived from human saliva and found that the viability of the biofilms exposed to the bioactive glass was significantly reduced compared with biofilms exposed to a bio-inert glass. One of the authors recently showed that fine particulate bioactive glass (NovaMin<sup>®</sup>)

incorporated into a non-aqueous dentifrice was able to reduce the viability of planktonic bacterial cultures of *S. mutans*, *F. nucleatum*, *A. naeslundii* and *S. sanguis*. A 2-min. exposure to a 1:3 dilution of both 3% and 10% bioactive glass-containing dentifrice reduced the viability of these organisms by 4.5 log against *F. nucleatum* to total loss of viability of the *S. sanguis* culture.

A study conducted to determine the inflammatory properties of these 5 µm bioactive glass particulates was undertaken by Rectenwald et al. (2002), using a mouse intra-peritoneal model to activate macrophage activity. The results of this study showed that the bioactive glass particles actually attenuated the pro-inflammatory response against an endotoxin challenge. The results showed an up-regulation of interleukin (IL)-6 without a concomitant increase in either IL-1β or IL-10. The study included a toxic shock model that showed an attenuation of serum levels of tumour necrosis factor (TNF)-α of 60% in animals dosed with the bioactive glass particulates compared with controls. In a clinical study evaluating the anti-inflammatory effects of bioactive glass bone graft material placed in periodontal defects, Han et al. (2002) found that crevicular elastase production in crevicular fluid was significantly lower in bioactive glass-grafted sites compared with root planing and debridement sites. The authors concluded that the lower elastase levels were an indication of less tissue destruction and lower inflammation at the sites.

In the current clinical study, there were statistically significant reductions in both the PLI (16%) and GBI (58%). The abrasivity of both the test and control toothpastes used in this study was similar, and well within the acceptable range for daily-use dentifrices. If the results presented in this study were solely due to the mechanical removal of plaque, then one would have expected there to be no differences between the active toothpaste and the control toothpaste. The fact that there was a 16.5% reduction in PLI and an even greater percentage reduction in GBI for the active toothpaste suggests that the action was more than just mechanical. Eberhard et al. (2004) recently published the results of an experimental gingivitis clinical study using a slurry of bioactive glass particulates. While he did not see any significant reductions in microbial activity as a result of the

application of the bioactive glass particulate, there was an attenuation of the clinical signs of inflammation, and there was a reduction in the measured IL-1β, although no significance was given. Recently, Xu et al. (2004) reviewed the properties of triclosan-containing dentifrices and ascribed at least some of the benefit of reduction of gingivitis of the compound to anti-inflammatory properties.

In the current study, a prophylaxis was performed after baseline measurements to bring the plaque levels to a uniformly low state, ideally close to zero. The results after the 6-week evaluation showed a complete re-growth of plaque in the control group to baseline levels before prophylaxis. In the experimental group there was statistically less plaque at 6 weeks than at baseline, although the GBI was substantially reduced (on a percentage of baseline) when compared with the PLI. This difference in the overall changes in the indices further suggests that there might be some anti-inflammatory component to the experimental formulation that is responsible for the reduction in GBI compared with the plaque reduction.

The results of the current study demonstrated a significant reduction in gingivitis in a relatively short period of time. There were no adverse events reported with the use of the material. To our knowledge, this is the first time that a dentifrice that does not contain antibiotics and/or fluoride has been shown to have a therapeutic effect on gingival health. Further clinical studies are required to determine the long-term effectiveness of this new compound. Additional studies should also be undertaken to determine if there will be any build-up of microbial resistance, and whether the reductions seen in the bleeding index in this study are due to a modification of the plaque composition either in amount or species of bacteria, or merely a reduction in the overall plaque level. While plaque presence has almost universally been associated with the development of gingivitis, it may be that plaque can exist without the progression of the disease.

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### Clinical Relevance Statement

The control of plaque in the maintenance of gingival health has been well established. Rigorous self-performed plaque control over long periods of time is known to reduce the levels and composition of the sub-

gingival bacteria. The relationship between bacteria, plaque and gingivitis in recent years has led to a focus on anti-microbial therapies for the treatment of gingivitis and periodontitis. This paper introduces the clinician to a compound that has

been used in bone and tissue regeneration, and that possesses inherent anti-microbial properties that may prove useful in the treatment of gingivitis.