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A randomised clinical trial to determine the effect of a toothpaste containing enzymes and proteins on gum health over 3 months

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ABSTRACT

Objectives: This study tested the efficacy of a toothpaste containing enzymes and proteins reflecting those naturally occurring in saliva which play an important role in maintaining bacterial balance to improve gingival health condition and reduce supra-gingival plaque formation over a period of 13 weeks as compared to a commercial control toothpaste.
Methods: This study was a double-blind, randomised, parallel group, 3 month home use study in healthy volunteers. Non-smokers with a mean modified gingival index (MGI) score of between 2.00–2.75 and at least 20 natural teeth, a minimum of 5 teeth in each quadrant were enrolled in the study. At screening, participants underwent a dental prophylaxis and were issued with a standard fluoride toothpaste and toothbrush to use for 4 weeks. After 4 weeks, participants demonstrating ongoing eligibility were assessed for gingival health and plaque score and randomised to either test or control toothpaste, which they used at home twice daily. After 13 weeks, gingival health and plaque were re-scored.
Results: 229 participants completed the study. There were no treatment associated adverse events. Plaque and gingival scores were significantly better in the test group as compared to the control group. Furthermore, in the test group plaque and gingival scores fell, while those in the control group rose over the 13 week period.
Conclusions: The test toothpaste containing enzymes and proteins demonstrated significant plaque and gingival benefit compared to the control toothpaste, and was well tolerated.
Clinical significance: Toothbrushing with the test product derived from naturally occurring enzymes and proteins had a clinical adjunctive improvement on gingival health compared to brushing alone with a commercially available fluoride toothpaste.

1. Introduction

Gingivitis is highly prevalent worldwide, with 46% of adults showing evidence of gingival bleeding and calculus (Community Periodontal Index score of 2), across all age categories [1]. In the UK, the most recent figures from the Adult Dental Health Survey 2009 demonstrated that 83% of adult participants exhibited poor oral health, and 50% of participants had sextants with periodontal pocketing of 4 mm or more [2]. This figure is similar to global estimates with current figures from the WHO Global Oral Health Data Bank showing that 46% of 35–44 year olds have evidence of periodontitis [1]. Severe periodontitis is the sixth most prevalent human disease, according to the 2010 global burden of diseases study, with a standardized prevalence of 11.2% [3].

Whilst gingivitis does not always progress to periodontitis, evidence to date has shown that in the majority of cases if gingival inflammation is prevented, periodontitis is prevented [4]. Gingivitis and periodontitis are derived from the same inflammatory disease with chronic gingival inflammation being the response to the presence of microbial biofilms. The same microbial biofilms are also considered to be the key risk factor for the onset of periodontitis, or its progression in treated patients [5]. Periodontitis has been shown to have a negative impact on oral health quality of life [6,7] and if left untreated, is a major cause of tooth loss [8,9]. This highlights the importance of effective treatments for the control of gingivitis, which unlike periodontitis, is reversible.

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One of the most significant risk factors for gingivitis is poor oral hygiene which results in the accumulation of plaque [10,11]. Dental plaque is a diverse biofilm that develops as bacteria preferentially attach to surfaces when the environmental conditions are favourable. In the oral cavity the initial phase of adhesion allows microbes to accumulate in the salivary pellicle that forms on the clean oral surfaces such as teeth and gingivae [12]. The initial attachment is tenuous and reversible but within minutes the bacteria become irreversibly attached. The ensuing accumulation of bacteria results in the formation of extracellular polymers which create a sticky hydrated matrix that holds the cells in close proximity that is difficult to penetrate and/or remove [13]. Over time the dental plaque biofilm develops into a complex structure which in health has been shown to provide benefits to the host such as resisting colonization by pathogens [14]. However, if dental biofilms are not regularly dispersed or disrupted by self-performed oral hygiene measures, they become dysbiotic as local conditions favour the emergence of pathogenic species. Bacteria of a given species can be present with a variety of phenotypes from rapid growth to dormant within the same biofilm. Ultimately an environmental shift occurs, resulting in gingival inflammation changes that favour periodontal pathogens [5,13]. So great is the importance of plaque control measures to contribute to the oral health status of an individual that they have been emphasized in all workshops on periodontology [15]. Treatments to prevent or resolve gingivitis are therefore focussed on improving oral hygiene and reducing dental plaque [16].

Oral hygiene should be practiced daily at home and treatments should be simple to improve chances of patient compliance. The bedrock of plaque removal is achieved by mechanical toothbrushing, twice daily, toothbrushing being widely promoted and playing a pivotal role in the prevention of periodontal diseases [17]. A recent meta review concluded that both manual and power brushes are effective at plaque removal, with average reductions in baseline plaque levels of 42% and 46%, respectively [18]. However, although the use of a toothbrush and fluoridated toothpastes is almost universal, the majority of the population do not clean their teeth thoroughly enough to prevent plaque accumulation [15], questioning the clinical effectiveness of mechanical oral healthcare interventions alone in managing gingivitis. This is supported by a number of studies [19–22] and systematic reviews [23]. The adjunctive use of an antibacterial agent for plaque control is therefore of benefit in those participants who are not able to effectively debride the oral surfaces of supragingival biofilms using mechanical procedures alone [24]. When used in combination with an adjunctive agent, toothbrushing reduces the amount of biofilm and disrupts its structure, allowing for a more effective action of the adjunctive formulation [25].

A number of adjunctive agents for plaque control and prevention of gingivitis have been developed, either incorporated into the toothpaste or used as a mouthrinse. In a comprehensive systematic review and meta-analysis by Serano et al [24] evidence for the efficacy of toothpaste formulations containing stannous fluoride (SnF_2), triclosan/copolymer or chlorhexidine (CHX) as antiplaque agents is provided. Evidence for the efficacy of triclosan/zinc citrate toothpastes was also reported in agreement with a previous review [26]. However, the relative merits of oral care products should also consider adverse effects which have been reported for some agents shown to be effective for plaque control. It is well-known that CHX causes extrinsic staining [27], and cetyl pyridinium chloride (CPC), SnF_2 and essential oil based formulations have also been shown to result in tooth staining [28–30]. In addition, triclosan, CHX and essential oil mouthrinses have also been reported to cause irritation of the oral soft tissues [31–33].

Saliva is known to contain proteins including enzymes and peptides which have antibacterial effects and are early responders of the innate immune system targeting invading pathogens [34,35]. Together with good oral hygiene it is believed that these antibacterial proteins and peptides keep the levels of pathogenic bacteria low [36]. A fluoride containing toothpaste utilises some of the salivary proteins with proven antibacterial effects, its formulation is designed to confer antiplaque activity. Three of the proteins contained in this toothpaste exhibit enzyme activity. Amyloglucosidase and glucose oxidase work together to produce hydrogen peroxide from polyglucans, and lactoperoxidase in the presence of sufficient hydrogen peroxide, catalyses the conversion of thiocyanate to hypothiocyanite. Both hydrogen peroxide and hypothiocyanite are antibacterial compounds which have been shown previously to target periodontal pathogens [37,38]. Other antibacterial proteins include immunoglobulins which provide protection against infection, lactoferrin which is a chelator of iron and inhibits the metabolic activity of several oral pathogens, and lysozyme which hydrolyses bonds in the peptidoglycan layer of bacterial cell walls [35,36,39]. It has been shown that treatment with this toothpaste containing these naturally antibacterial enzymes and proteins increases levels of salivary hypothiocyanate, hydrogen peroxide and lysozyme, and decreases the viability of biofilms of oral bacteria in vitro [40]. Thus this toothpaste strengthens the natural antibacterial systems in the oral cavity, and in a recent randomised clinical trial was shown to promote the relative abundance of bacteria associated with periodontal health and decrease those associated with periodontal disease [38]. In addition, this toothpaste contains the surfactant Steareth-30, a non ionic polyethylene glycol ether of stearic acid [41] which is a very effective emulsifier that stabilises dispersed systems [42], whereas the majority of toothpastes contain sodium lauryl sulphate (SLS) which has been shown to cause gingival sloughing in some individuals [43].

The prevalence figures for gingivitis [2] together with the adverse effects reported in some individuals for some current formulations indicate that the development of new products for the prevention and treatment of this disease is warranted. It is anticipated that a toothpaste formulation based on antibacterial components found naturally occurring in saliva, together with fluoride and a neutral surfactant in the form of Steareth 30 instead of SLS will provide plaque control that is well tolerated by most patients.

Ideally plaque inhibitory and antiplaque activities of a given formulation should be proven in a home-use, long-term study, randomised clinical trial (RCT). Further, the agent should be used as an adjunctive to mechanical plaque control, as described by the conclusions of the Council of Dental Therapeutics in 1986. The aim of the present study was to determine the efficacy of a toothpaste containing natural enzymes and proteins with toothbrushing as compared to a control fluoride toothpaste with toothbrushing, in the control of dental plaque over a 3 month time period.

2. Materials and methods

2.1. Study design and conduct

This study was a double-blind, randomised, parallel group, 3 month home use study in 229 healthy volunteers. The study investigated the relative efficacies of two of mechanical and adjunctive agent plaque control regimens to improve the gingival condition. Regimen one comprised a toothpaste containing proteins and enzymes (Zendium™-1450 ppm Sodium fluoride, lactoperoxidase, lactoferrin, colostrum, amyloglucosidase, glucose oxidase, lactoperoxidase and potassium thiocyanate) with toothbrushing. Regimen two comprised a commercial control fluoride toothpaste (Sensodyne Pronamel® – Sodium fluoride 1450 ppm) with toothbrushing. The study was given ethical approval by the South West-Exeter Research Ethics Committee (Reference: 16/SW/0190) and was conducted to Good Clinical Practice Guidelines as described by the Declaration of Helsinki. The study was registered on the ClinicalTrials.gov database (identifier: NCT03027908). Participant recruitment, screening, treatment and clinical assessments were carried out at the study site, a UK Dental School.

Volunteers who expressed an interest in taking part in the study were invited to the study site for screening and allocated a unique screening number. To ensure an approximately equal number of males

and females were enrolled in the study, an approximately equal number were screened and the number of each gender fulfilling the eligibility criteria monitored. Those participants who were happy to take part in the study gave written informed consent and were then screened for eligibility, the study dentist taking a medical history, conducting an oral soft tissue examination (OST), and performing Modified Gingival Index (MGI), Bleeding Index (BI) and Plaque Assessments on all scoreable teeth. Eligible participants were healthy adults 18 years or over with a mean MGI score of between 2.00–2.75 and at least 20 natural teeth, with a minimum of least 5 teeth in each quadrant. Exclusion criteria were pregnancy or breast feeding, antibiotic or antimicrobial treatment within 4 weeks of the screening appointment, antihistamine medication within 24 h of the screening appointment, obvious untreated caries, significant periodontal disease, partial or complete dentures, oral piercings, ongoing orthodontic treatment and smoking, including e-cigarettes. Vegans and vegetarians were also excluded as one of the toothpastes contains ingredients derived from milk and egg sources. Participants that satisfied the inclusion and exclusion criteria were enrolled onto the study, given a professional dental prophylaxis and provided with a conventional cosmetic silica fluoride toothpaste and a toothbrush to use at home, twice daily for 4 weeks. In addition, participants were asked to refrain from the use of dental products (other than those provided) during the pre-treatment and test phases of the study, this included interdental cleaning aids, and participants were also asked to abstain from any professional cleaning. Compliance with all study restrictions was checked at the beginning of each visit to the study site.

After 4 weeks, participants returned to the study site for the baseline visit having abstained from toothbrushing or using any oral hygiene products (mouthwashes, medicated floss or chewing gum) from midnight the evening before, and having refrained from eating or drinking for at least one hour before their study appointment. To confirm ongoing eligibility for the study, a medical history review, OST exam, MGI, BI and plaque assessments were repeated on all scoreable teeth. At this appointment any participant with a single MGI score of 3 was withdrawn from the study. Following baseline assessments, participants were randomised utilising gender stratification, to one of the two toothpastes according to the randomisation schedules prepared by the study statistician.

Randomisation numbers were assigned by study staff in ascending numerical order as participants were deemed eligible to continue in the study. Two randomisation schedules were provided, one for males and the other for females, each schedule designed so that an equal number of participants of the gender would be allocated to test or control if a similar number of each were enrolled. For female participants randomisation numbers were prefixed by an 'F' and for male participants the prefix was 'M'. To maintain blinding, randomisation and product dispensing were undertaken in an area completely separate to that in which the study dentist undertook the clinical assessments. Product dispensing was carried out by additional study staff, the study dentist was only told the participant's randomisation number. To ensure participants were blind to the treatment they were receiving their study toothpaste was provided in a plain white tube labelled with a code which bore no resemblance to the product names or the words test or control. Participants were also given a toothbrush (the same for both groups) and instructed to use the allocated product at home, twice a day for the duration of the study.

After 13 weeks participants returned to the study site for their final visit having abstained from toothbrushing or using any oral hygiene products (mouthwashes, medicated floss or chewing gum) from midnight the evening before, and having refrained from eating or drinking for at least one hour before their study appointment. At this final appointment a medical history review, OST exam, MGI, BI and plaque assessments were repeated on all scoreable teeth, and all study products were returned to the study site.

2.2. Clinical measurements

At each visit, three outcome variables were recorded for each scoreable tooth. The analyses of efficacy of the toothpastes with toothbrushing were based on the scores averaged over all the sites of the scoreable teeth. The primary outcome measure was the MGI [44] scored on a 5 point scale at 4 sites per tooth (buccal and lingual/palatal marginal gingivae and interdental papillae). Score 0 = normal (absence of inflammation), score 1 = mild inflammation (slight change in colour, little change in texture) on any portion of the gingival unit, score 2 = mild inflammation of the entire gingival unit, score 3 = moderate inflammation (moderate glazing, redness, oedema, and/or hypertrophy) of the gingival unit, score 4 = severe inflammation (marked redness and oedema/hypertrophy, spontaneous bleeding, or ulceration) of the gingival unit. The BI [45] was scored on a 3 point scale at four gingival sites per tooth (distobuccal, midbuccal, midlingual, and mesiolingual); 0 = absence of bleeding after 30 s, 1 = bleeding after 30 s, 2 = immediate bleeding on probing. The third outcome measure used was the Plaque Index (Modified Quigley and Hein) [46] which was scored on a 6 point scale at 3 buccal and 3 lingual sites per tooth after the teeth had been disclosed with liquid disclosing solution. Score 0 = no plaque, score 1 = separate flecks of discontinuous band of plaque at the gingival margin, score 2 = thick (up to 1 mm), continuous band of plaque at the gingival margin, score 3 = band of plaque wider than 1 mm, but less than one third of surface, score 4 = plaque covering one third or more, but less than two thirds surface, score 5 = plaque covering two thirds or more of surface.

2.3. Sample size and statistical analysis

The study was designed to recruit a minimum of 240 consenting participants who satisfied the eligibility criteria so that at least 200 participants (100 each group) completed the study. This sample size gives 80% power to detect a difference of 0.4 times the within-groups standard deviation of the whole-mouth MGI score between the experimental and control groups at 13 weeks using a two-sided independent sample *t*-test at the conventional 5% alpha level.

At each time point, summary statistics were calculated for the test toothpaste group and the control toothpaste group, for mean scores for the three measures over all scoreable teeth. Analysis was carried out both within-groups and between-groups. In the within-groups each of the above measures were compared between baseline and 13 weeks. The mean change is reported, with a 95% confidence level and associated p-value as determined by paired *t*-test In the primary, between-groups, analysis, each of the above measures was compared between the experimental group and the control group, at 13 weeks, using an analysis of covariance model with 2 factors, treatment and gender, using the corresponding baseline value as covariate. The mean difference is reported, with a 95% confidence level and associated p-value.

3. Results

Recruitment commenced in October 2016 and the study was completed in April 2017. Participant flow through the study is shown in Fig. 1. At screening 245 participants of the 261 screened were enrolled onto the study. At baseline 2 participants were lost to follow up, one withdrew and 239 participants who demonstrated continued eligibility were randomised. Following randomisation 10 participants withdrew from the study so that 229 participants completed the study. There were 7 non-treatment related adverse events recorded during the study period. Participant demographics are shown in Table 1. The two treatment groups were well balanced for age and gender, though ethnic minorities were more strongly represented in the group allocated to the test fluoride toothpaste containing natural enzymes and proteins.

Changes from baseline to 13 weeks for both treatment groups for MGI, BI and plaque score are shown in Table 2. In the control group

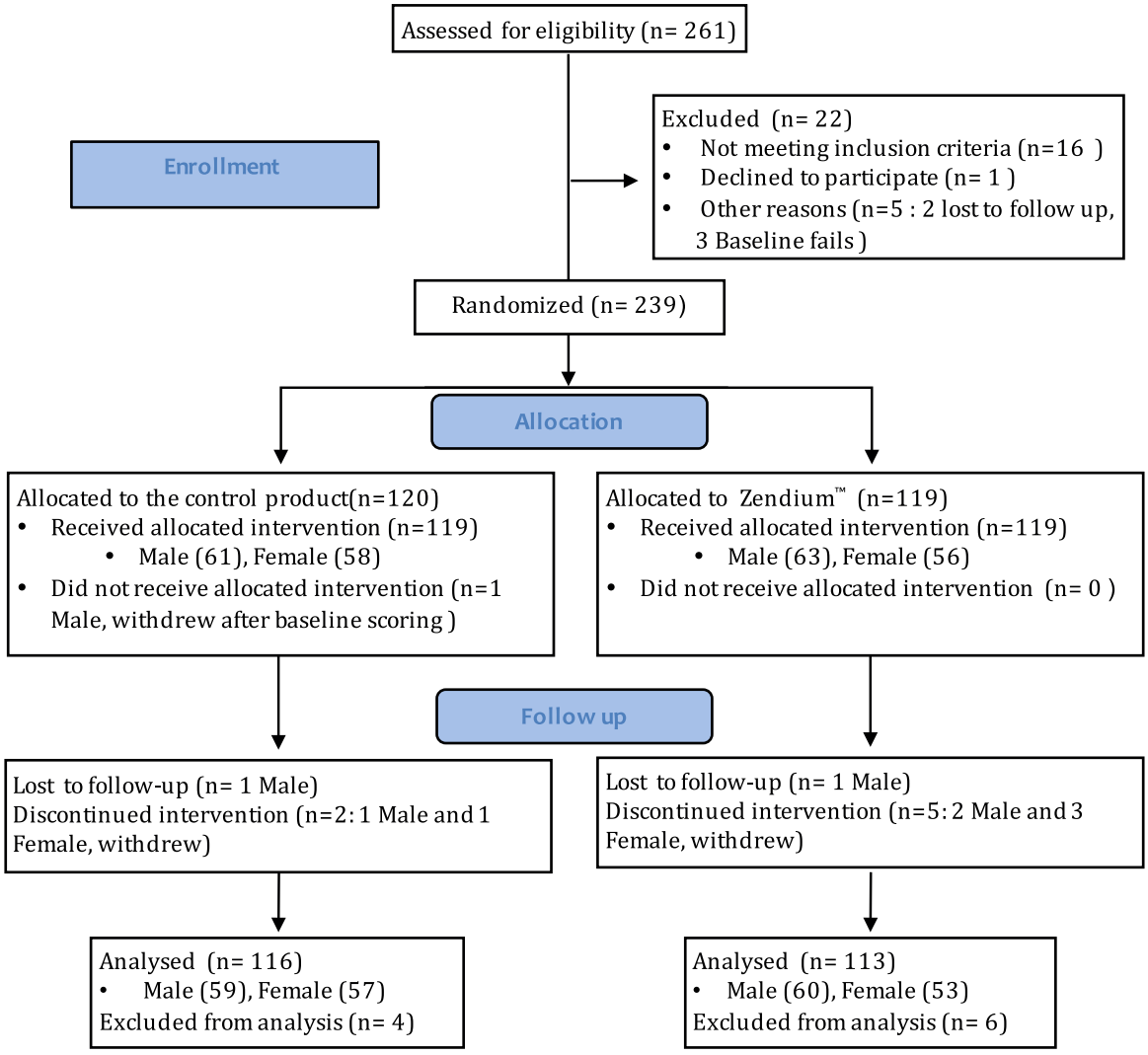

Fig. 1. Participant flow through the study.

Table 1
Participant demographics.

	All participants (n = 229)	Fluoride Control® (n = 116)	Zendium™ (n = 113)
Mean age (min, max)	32.6 (18, 74)	32.2 (19, 74)	33.0 (18, 68)
Gender			
Male	119	59	60
Female	110	57	53
Ethnic group			
Asian	23	9	14
Black	3	1	2
Caucasian	194	104	90
Hispanic	1	1	0
Mixed	8	1	7

(commercially available fluoride toothpaste) all three measures had increased by 13 weeks, with the increase in MGI from 1.657 to 1.744, significant. By contrast, in the test group who used the toothpaste containing natural enzymes and proteins all three measures decreased significantly, with the change for MGI and plaque score highly significant, reducing from 1.627 and 2.223 to 1.404 and 2.112, respectively.

Comparing the two groups following 13 weeks of product use, all three measures showed a marked superiority for the test toothpaste (containing natural enzymes and proteins) treatment over the control toothpaste as shown in Table 3. For each measure, the difference was significant before and after adjustment of the means for the baseline score and gender.

4. Discussion

The present study compared a test fluoride toothpaste, containing naturally occurring enzymes and proteins, with a commercially available fluoride control toothpaste. The study demonstrated that toothbrushing with the test product resulted in significantly better gingival and plaque scores compared to toothbrushing with the control toothpaste after 13 weeks. Furthermore, gingival and plaque scores increased from baseline in the control group, and decreased for all measures, gingival, plaque and bleeding scores, in the test group.

While antibacterial adjuncts can be effectively delivered in mouthrinse formulations, these must be administered in addition to the normal toothbrushing regimen with toothpaste. This may be considered to be a burden of home dental care, and can easily be omitted. Furthermore, there is the consideration of chemical interaction between oral healthcare products which may result in the negation of their respective benefits [47]. The optimal way to deliver antibacterial adjuncts is within toothpastes, as this reduces the number of activities required on the part of the patient as well as reducing the risk of potential chemical interaction. This is achieved by reducing the constituents to proven and prescribed toothpaste formulations where no

Table 2
Change in MGI, BI and plaque score from Baseline to 13 Weeks.

	Mean change	Lower CL*	Upper CL*	t-ratio	p-value**
MGI					
control toothpaste	0.087	0.043	0.131	3.92	< 0.001
toothpaste with proteins and enzymes	−0.223	−0.268	−0.178	−9.90	< 0.001
BI					
control toothpaste	0.032	−0.003	0.067	1.80	0.075
toothpaste with proteins and enzymes	−0.043	−0.080	−0.007	−2.34	0.021
Plaque					
control toothpaste	0.004	−0.038	0.047	0.20	0.841
toothpaste with proteins and enzymes	−0.111	−0.162	−0.060	−4.30	< 0.001

* 95% confidence level.
** Paired *t*-test A negative value indicates improvement while positive values indicate worsening.

Table 3
Comparisons between treatment groups at 13 weeks.

	Difference	Standard error	Lower CL*	Upper CL*	t-ratio	p-value
MGI						
Adjusted	0.329	0.029	0.272	0.386	11.33	< 0.001**
BI						
Adjusted	0.081	0.024	0.034	0.129	3.39	< 0.001**
Plaque						
Adjusted	0.111	0.031	0.051	0.172	3.61	< 0.001**

* 95% confidence level.
** ANCOVA. Positive differences indicate superiority of the test product over the fluoride control.

interactions exist. Further, when antibacterial adjunctive agents are delivered in combination with the mechanical action of brushing, the physical disruption of the biofilm improves the access of the antibacterial adjunctive agent to the gingival margin [13].

The incorporation of active ingredients into toothpastes is not however without its problems, some agents, for example, CHX are susceptible to inactivation with SLS [47], the most common surfactant used in toothpastes [41]. To date, evidence from systematic reviews suggests that only toothpastes containing stannous fluoride (SnF) with or without sodium hexametaphosphate (SHMP), toothpastes containing triclosan/copolymer (tric/cop) and toothpastes containing CHX provide statistically significant improvements in gingival bleeding and plaque indices as compared to negative controls [17,24,26,48,49]. There is also some evidence that triclosan/zinc citrate toothpastes are beneficial for gingival health [26]. The demonstration that the fluoride toothpaste containing naturally occurring enzymes and proteins tested in this study is also able to reduce gingival and plaque indices, performing significantly better than a fluoride control toothpaste, is therefore of importance. The changes in the gingival indices over 13 weeks observed for this test toothpaste are in line with those found for the toothpastes for which significant efficacy for gingival indices over control pastes has been demonstrated over 26 weeks [24].

The mechanism of action of the fluoride containing test toothpaste for reducing gingival indices is thought to be via the naturally occurring enzymes and proteins in its formulation that enable it to augment the naturally occurring salivary peroxidase system, one of the major defence mechanisms of the oral cavity. In the presence of hydrogen peroxide produced by oral bacteria salivary peroxidase catalyses the oxidation of thiocyanate (SCN^-) to hypothiocyanite ($OSCN^-$) which inhibits the growth of bacteria [35,50]. To enhance this natural system, as well as lactoperoxidase, the test toothpaste contains the enzymes amyloglucosidase and glucose oxidase which work together to increase the levels of hydrogen peroxide and thus the conversion of SCN^- to OSCN- by lactoperoxidase. In addition, the toothpaste contains the salivary enzymes lysozyme and lactoferrin. These proteins have both bacteriocidal and bacteriostatic activities and it has been shown that the actions of the three antimicrobial agents lactoperoxidase, lactoferrin and lysozyme are additive [51]. Further, it has been demonstrated that these enzyme components can be immobilised on the enamel pellicle [52].

Support for the ability of the test toothpaste to enhance natural salivary defences against pathogens has been obtained recently in a randomised controlled trial where it was demonstrated that this combination of enzymes and proteins promoted a shift in the oral microbiome from bacteria associated with periodontal disease to those associated with periodontal health after 14 weeks of treatment [38]. An earlier study also provided support for the antimicrobial activity of such enzyme based toothpastes, demonstrating an increase in resting levels of $OSCN^-$ following 1 month of twice daily use [53]. Further, in an article included in this issue [40] a series of studies demonstrated that the test toothpaste used in the present study boosted levels of $OSCN^-$, hydrogen peroxide and lysozyme in saliva in vitro, interfered with the membrane integrity of *Streptococcus mutans* and Fusobacterium nucleatum, and reduced oral bacterial viability in both single species and multi species biofilm models.

Few studies have examined clinical outcomes following treatment with toothpastes containing naturally occurring enzymes and proteins, but in a 4 day plaque regrowth study a toothpaste with a similar enzyme and protein formulation performed significantly better than a placebo toothpaste [54]. Similarly, in a more recent study it was demonstrated that gingivitis scores decreased over the 8 weeks of the study following treatment with another similar toothpaste containing natural enzymes and proteins, although no difference between this toothpaste, and the control toothpaste was detected [55]. However, unlike the test toothpaste, the control toothpaste used in the study contained SLS which has been shown to have an antibacterial effect in vitro and inhibit plaque re-growth in vivo as compared to a high fluoride rinse [56,57] and so might be expected to have a positive effect on gingival health. The data obtained in the present study is also supported by the findings of Pedersen et al. [58] who demonstrated that in a study of age and gender matched participants, those who had used a similar toothpaste containing enzymes and proteins during the previous year had significantly lower gingival and bleeding scores than those using an alternative fluoride toothpaste during this time.

In the present study, enrolled individuals were provided with a professional prophylaxis at screening to reduce the level of existing gingivitis, following which, participants were given a commercially available control fluoride toothpaste to use in place of their own for a 4 week run in period. The control fluoride toothpaste, similar to the test toothpaste, did not contain SLS. No further dental prophylaxis was given at baseline. This novel approach was designed to reduce the Hawthorne effect [59] whereby participants perform study related tasks, such as oral hygiene to a higher level than normal, because they are participating in a clinical trial. This methodology has the advantage of engendering the participants' toothbrushing style in the treatment phase, to be similar to their normal brushing habits, reducing the

Hawthorne effect. In addition, where a dental prophylaxis is carried out at baseline, it may be difficult to determine whether the plaque control agent under investigation is effective at prevention, or whether gingivitis therapy can be attributed.

This study was designed to investigate the preventive effect of a fluoride toothpaste containing enzymes and proteins with regards to gingival inflammation. As gingival indices and plaque scores decreased from baseline, this suggests it may be beneficial to consider undertaking six month studies to investigate its longer term efficacy. In addition, the choice of a control fluoride toothpaste, with no benefit for gingival inflammation reduction, as compared to a toothpaste with confirmed efficacy for the treatment of gingivitis, means that it is not possible to say that the toothpaste with enzymes and proteins is better than market leaders in the field, but rather that the test paste is superior to the conventional over the counter paste evaluated. Further studies to test the enzyme based toothpaste against toothpastes with confirmed efficacy for gingivitis are required.

The rationale for choosing the control toothpaste in the present study was that in the marketplace it was readily available, SLS free and non-foaming to be similar in this respect to the test toothpaste which is designed as an effective formulation for gingivitis for those who find SLS based products can result in oral soreness [60]. While oral soreness and transient soft tissue lesions have been reported by healthy volunteers in response to SLS based formulations [43,60], it is recognised that in the main, problems are encountered in those who suffer from aphthous ulcers or recurrent aphthous stomatitis [61,62] suggesting that this population in particular would benefit from a non-SLS gingivitis paste. The test toothpaste was well tolerated during the study, a finding considered to be due to its formulation being based on components that are found naturally in saliva and the use of the mild surfactant Steareth-30 which recent in vitro and in vivo studies caused minimal gingival sloughing as compared to SLS [43]. However, patients were not asked directly to rate their experience following use of this toothpaste in terms of satisfaction with the taste or any oral discomfort, this is a study limitation that should be addressed in future clinical studies.

5. Conclusions

In conclusion, this study demonstrates that toothbrushing with a toothpaste formulation containing naturally occurring proteins, enzymes and Steareth 30, is effective at preventing gingivitis compared to toothbrushing with a commercially available fluoride toothpaste, having good antiplaque activity in the absence of side effects.

Conflict of interest and funding statement

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