

Antimicrobial effects of *o*-cymen-5-ol and zinc, alone & in combination in simple solutions and toothpaste formulations

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Antimicrobials are added to toothpastes to deliver dental plaque/gingivitis benefits. **Objectives:** This study aimed to evaluate antimicrobial effects of an *o*-cymen-5-ol/zinc system. **Methods:** *o*-Cymen-5-ol and zinc gluconate minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined against *Streptococcus mutans*, *Actinomyces viscosus*, *Porphyromonas gingivalis*, *Fusobacterium nucleatum* and *Candida albicans*. Synergy was investigated by checkerboard MIC/MBC; inhibition of *P. gingivalis* protease activity and *S. mutans* glycolysis were investigated. Slurried toothpastes containing the system were assessed in kill time assays against *S. mutans* and *E. coli*. **Results:** *o*-Cymen-5-ol MIC was between 1.7 mM to 3.4 mM; MBC was 3.4 mM to 6.7 mM. Zinc gluconate MIC was 2.8 mM to 11 mM; MBC was between 11 mM and >44 mM. The two agents in solution showed synergy (FICI ≤ 0.50) against *P. gingivalis* and *F. nucleatum*, with MIC of 0.42 mM/0.69 mM for *o*-cymen-5-ol/zinc gluconate, respectively. Zinc inhibited glycolysis and protease to a greater degree than *o*-cymen-5-ol; glycolysis inhibition by the two agents was additive. *o*-Cymen-5-ol/zinc chloride in toothpaste showed greater effects than placebo (120s log₁₀ kill = 7.35 ± 0.40 and 4.02 ± 0.40, respectively). **Conclusions:** The zinc/*o*-cymen-5-ol system has direct antimicrobial effects and inhibits oral disease-related processes. Synergistic effects were seen against anaerobes. A system combining *o*-cymen-5-ol and zinc shows properties desirable for incorporation in toothpastes.

Key words: Antimicrobial, gingivitis, dental plaque, Toothpaste, zinc, *o*-cymen-5-ol

The human mouth is sterile at birth, but, rapidly becomes colonised by a diverse microflora¹, typically with hundreds of species in any one adult individual's mouth² selected from potentially several thousand species found across human populations; the debate continues as to whether such numbers in an individual can comprise many thousands of microbial species³. The oral microflora exists in an intimate relationship with the host, with particular groups of bacteria associated with specific oral habitats^{4,5}. In spite of a wide range of dietary and environmental challenges, the oral microflora remains broadly similar across human populations. This stability has been termed microbial homeostasis^{6,7}, and is assumed to result from the comprehensive biochemical capabilities of the entire oral microflora, together with the intricate web of metabolic interactions between members of the microflora^{8–11}. Significant environmental disruption, for example by antibiotics, often allows overgrowth of smaller or insignificant members of the oral microflora, resulting in conditions such as oral candidiasis^{12,13}. With these exceptions, the microflora usually exists in a benign and stable relationship with the host. However, the oral microflora is also key to the most

common chronic diseases affecting humans, dental caries and gingivitis.

Dental caries is microbially-driven, by generation of lactic and other acids from dietary carbohydrate by a range of microbes, and is associated with excessive and frequent sugar consumption^{14,15}. Most attention has focussed on the role of mutans-streptococci, because of their acidogenic, aciduric and adherent properties¹⁶ and association in earlier studies with sugar consumption^{17–20}, and disease susceptibility²¹. More recent evidence suggests that a large number of microbial taxa other than mutans-streptococci can also generate significant quantities of acid^{22,23} and are associated with caries²⁴. The specific bacteria implicated in gingivitis remain unclear. However, it is clear that excessive accumulation of dental plaque and associated by-products at the gum margin produces gingivitis²⁵, which in turn may lead to increased microbial nutrition (via increased gingival crevicular fluid flow, blood etc) and further microbial proliferation. Earlier studies suggested the involvement of general groups of organisms (Gram-negative anaerobes, specific genera from the 'orange' or 'red' complex)²⁶. More recent studies, however, suggest a much broader aetiology, with greater similarities within a

subject than between diseased and non-diseased sites^{27,28}. Periodontitis is much rarer than gingivitis, and while initiated by bacterial infection also appears to be linked to immunoinflammatory pathways²⁹, although prolonged pre-existing gingivitis is also considered a risk factor³⁰.

Oral care products are designed to combat the two key microbially-mediated diseases, caries and gingivitis. The beneficial effects of topical fluoride in toothpastes have driven a dramatically reduced caries incidence in the last 30–40 years³¹. Fluoride appears to reduce caries principally via effects on enamel de- and remineralisation³² although it can also have potentially caries-relevant antimicrobial effects, notably on bacterial metabolism, and especially on glycolytic and acid-generating pathways³³. Gingivitis, in contrast, may usually be prevented or treated by a diligent oral hygiene regime³⁴. The major beneficial effect of tooth brushing on gingivitis thus rests on the mechanical removal of dental plaque, with this process assisted by toothpaste formulation excipients such as abrasives and surfactants. However, most of the population fail to carry out meticulous oral hygiene, and thus the incorporation of additional antimicrobial actives in toothpastes as an adjunct to mechanical plaque control has been proposed^{35,36}. Products must tread a relatively fine line, termed an antimicrobial paradox³⁷, wherein the aim is to suppress the plaque to the extent required to prevent or reduce gingivitis, but not to eliminate the oral microflora or to disrupt it significantly. Such agents must meet a number of important criteria which were recently reviewed³⁸. The most widely used antimicrobial agent in toothpastes is Triclosan. In clinical trials, significant gingivitis benefits were seen when Triclosan was formulated with an additional antimicrobial agent – zinc citrate³⁹, or with a co-polymer, Gantrez⁴⁰, which improves oral Triclosan retention. Triclosan is, however, currently under review by the US Food & Drug Administration and Environmental Protection Agency⁴¹. Stannous fluoride in toothpaste delivers anti-gingivitis efficacy⁴² and is recognised in the tentative FDA anti-gingivitis monograph⁴³. Stannous fluoride does, however cause staining problems⁴² and adversely affects the taste of toothpastes. The essential oils combination of eucalyptol, menthol, methyl salicylate and thymol is recognised in the FDA monograph to deliver anti-gingivitis efficacy in mouthwash⁴³, and has also been reported to be effective in toothpaste against gingivitis⁴⁴. However, thymol in particular, causes a rather polarising, burning sensation, which limits acceptability for family-orientated toothpastes. Zinc salts have been advocated based on a number of beneficial antimicrobial effects the cation can deliver. Zinc inhibits a range of microbial enzyme systems, including a range of glycolytic enzymes in streptococci, with concomitant increased sensitivity of the organisms to acid⁴⁵. In two typical oral anaerobes, *Fusobacterium*

nucleatum and *Prevotella intermedia*, a range of enzymatic processes are inhibited by zinc, including catabolism of amino acids, sugars and peptides, as well as oxidative metabolism⁴⁶. Zinc can also deliver beneficial effects for bad breath: reducing breath concentrations of volatile sulphur compounds (VSCs) associated with halitosis^{47,48} as well as having direct⁴⁹ and indirect⁴⁶ inhibitory effects on anaerobe mediated-VSC production.

There is thus a clear need for novel antimicrobial systems which overcome some of the issues associated with Triclosan, stannous fluoride and essential oils, and that can deliver antimicrobial effects in a product with a taste profile acceptable to the whole family. This paper discusses the antimicrobial testing carried out on a novel toothpaste system, incorporating *o*-cymen-5-ol (a tasteless isomer of thymol) and zinc chloride. The agents in this system have been tested, alone and in combination, in a series of standard antimicrobial assays, in simple solutions and in whole toothpaste formulations. Further, the agents have been evaluated for their inhibitory effects on key metabolic processes – acid production by *Streptococcus mutans* and protease activity of *Porphyromonas gingivalis*.

MATERIALS AND METHODS

Bacterial strains and growth conditions

Strains were sub-cultured from –80°C or freeze dried stocks. *Streptococcus mutans* NCTC 10449 and *Streptococcus mutans* UA159 were then maintained on blood agar at 37°C in an aerobic incubator. *Actinomyces viscosus* NCTC 10951, *Fusobacterium nucleatum* NCTC 10562 and *Porphyromonas gingivalis* ATCC 53978 were maintained on blood agar anaerobically at 37°C. *Candida albicans* NCPF 3179 was maintained on Sabouraud's Dextrose agar (Oxoid) at 25°C in an aerobic incubator.

Minimum inhibitory concentration and minimum bactericidal concentration assays

The minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC), were measured using methods described by the National Committee for Clinical Laboratory Standards (NCCLS)⁵⁰ broth microdilution method with some modifications including: use of flat bottomed 96-well microtitre plates (Corning 3370, FisherScientific, Loughborough, UK), sterile deionised water used as diluent, brain heart infusion broth used in place of Muller-Hinton broth and 100 µl of inoculum added to wells. *o*-Cymen-5-ol stock solution was prepared at 0.4%w/w (26.67 mM) in 50%v/v DMSO. Zinc gluconate stock solutions were prepared in water. Zinc

gluconate was used in simple solution experiments in place of zinc chloride to ensure adequate solubility and stability, and to avoid pH effects, and was made in equivalent zinc molarity to toothpaste concentrations. Briefly, 2-fold serial dilutions of *o*-cymen-5-ol and zinc gluconate were prepared in sterile deionised water at a volume of 100 µl in the wells of a polystyrene 96-well microtitre plate. This was diluted to give final concentrations of *o*-cymen-5-ol in MIC wells from 0.0033 mM to 6.66 mM (0.00049 mg/ml to 1 mg/ml). The final concentrations of zinc gluconate ranged from 0.022 mM to 44.02 mM (0.00981 mg/ml to 20.1 mg/ml). The wells were inoculated with 100 µl of bacterial cell suspension in double strength BHI (Merck) at a final concentration of approx. 1×10^6 cells/ml. Control wells of water and uninoculated double strength BHI served as a reference and wells containing test organism in BHI were used as a growth control. The plates were incubated anaerobically for 48 hrs, (for *F. nucleatum* and *P. gingivalis*), and aerobically for 24–48 hours, (for *S. mutans*, *A. viscosus* and *C. albicans*), all at 37°C. Growth was assessed by measuring absorbance at 550 nm using a microplate spectrophotometer (SpectraMax M5, Molecular Devices, California, USA) and the lowest concentration inhibiting growth relative to uninoculated control was considered as the MIC. The MBC was measured by taking a 10 µl aliquot from two wells either side of the last well to show growth and plating onto Columbia blood agar (Biomérieux, Basingstoke, UK) followed by appropriate incubation. MBC was considered to be the lowest concentration showing no growth on blood agar after 48 hours incubation.

MIC checkerboard assay

To determine the lowest combinations of *o*-cymen-5-ol and zinc gluconate that were inhibitory to growth a checkerboard MIC/MBC method was used. Stock solutions were prepared as described above. Two-fold dilution series of *o*-cymen-5-ol and zinc gluconate at four times final concentration were prepared in sterile deionised water. Fifty microlitres of sterile water was added to wells A-H in columns 1–8 of a 96-well microplate. Fifty microlitres of the first *o*-cymen-5-ol dilution was added to wells A-H in column 1 and the doubling dilution series continued down to column 8. Zinc gluconate dilutions were added in a similar manner in wells of rows A-H in columns 1–8 to give 64 wells each containing a different concentration of either *o*-cymen-5-ol or zinc gluconate. The wells were inoculated with the test organism at a cell density of approx 1×10^6 cfu/ml in double strength BHI. Wells in column 11 contained 50 µl of double strength BHI in 150 µl sterile water and were used as a reference. Wells in column 12 contained 50 µl of test organism

in double strength BHI and 150 µl sterile water and were used as a growth indicator. Microplates were incubated at 37°C for 24–48 hours and the MIC was determined as above. MIC was defined as the lowest concentration of *o*-cymen-5-ol and zinc gluconate that inhibited growth of the test organism. The MBC was determined as above by plating a 10 µl aliquot of each test well in each row onto blood agar. The MBC was defined as the lowest combination of *o*-cymen-5-ol and zinc gluconate that failed to grow on blood agar. Synergy was defined conservatively as Fractional Inhibitory Concentration Index (FICI) ≤ 0.50 according to the criteria described by Odds⁵¹ and by Johnson *et al.*⁵² where:

$$\text{FICI} = \frac{\text{MIC}_{o\text{-Cymen-5-ol with Zinc gluconate}}}{\text{MIC}_{o\text{-Cymen-5-ol alone}} + \text{MIC}_{\text{Zinc gluconate with } o\text{-Cymen-5-ol}}} / \text{MIC}_{\text{Zinc gluconate alone}}$$

In principle, FICI ≤ 1.00 would indicate an increased activity of two agents in combination. However the criteria defined by Odds⁵¹ and by Johnson *et al.*⁵² also incorporate the need for a two-doubling-dilution difference in activity for *both* agents to indicate clear synergy.

Antibacterial activity of solutions and whole toothpastes

Toothpastes or solutions formulated to contain *o*-cymen-5-ol, zinc or the two agents combined were tested for antibacterial activity using the European suspension test⁵³ method with the following modifications; test was scaled down to accommodate a 2 ml deep well plate (Corning Code 3960) using 100 µl artificial saliva as interfering substance, 100 µl bacterial suspension, 800 µl of solution or toothpaste slurry (made to 120% of required final assay concentration) in hard water. *o*-Cymen-5-ol and zinc gluconate stock solutions were prepared in DMSO and water, respectively as above. Toothpastes were slurried to give dilutions of 1/4 or 1/6, and compared to a placebo product containing neither active but otherwise identical except for the absence of a buffer system. Contact times of 30 seconds and 120 seconds were used to simulate typical contact times during brushing. At these times a 100 µl aliquot of test mixture was taken from the test well and added to 900 µl of neutraliser containing Tryptone Soya Broth, 4% Tween 20 and 0.5% Lecithin (Oxoid, Code BO 1084J, Basingstoke, UK). The test mixture was allowed to neutralise for 10 minutes before being decimally serially diluted in 1% Peptone water with 0.85% Sodium chloride (Oxoid). Dilutions were plated onto Blood Agar number 2 (Oxoid, Basingstoke, UK) and incubated at 37°C anaerobically for 48–72 hours.

Protease inhibition assay

Solutions of *o*-cymen-5-ol and zinc gluconate were investigated for inhibition of *Porphyromonas gingivalis* tissue destructive arg-gingipain protease using the method described by Percival and colleagues⁵⁴ with the following modifications: assay was scaled down to fit in the wells of a 96-well microtitre plate, (Corning) and buffer used was phosphate buffered saline (PBS) (Sigma) at pH 7.49. *o*-Cymen-5-ol and zinc gluconate stock solutions were prepared in DMSO and water, respectively as above. Test wells of the microplate contained 160 µl of test solution diluted in assay buffer, 40 µl of the synthetic enzyme substrate DL- α -Benzoyl-DL-arginyl-p-nitro-anilide (BAPNA) (Sigma, Product Code B3133) and 20 µl *P. gingivalis* culture in BHI broth (grown for 48 hours). Controls used the same mixture as above but with test agent replaced with 160 µl of assay buffer. The assay was carried out in triplicate. The increase in absorbance was read every minute for three minutes at a wavelength of 405 nm using a microplate spectrophotometer. Results were presented as percent inhibition of protease enzyme activity [100- (change in OD of test agent/ change in OD of control) x 100].

Glycolysis inhibition assay

Assays of acid production under pH-stat conditions were carried out as described previously⁴⁵ for thick suspensions of cells of *S. mutans* UA159. Briefly, for the assays, cells were used from cultures in the early stationary phase of growth in BHI medium with excess

glucose so that the cells were fully acid-adapted. Stock solutions were prepared as above. Inhibitory effects on glycolysis of *o*-cymen-5-ol, zinc chloride and SLS were compared alone and in combination.

RESULTS

MIC and MBC

Data presented in Table 1 show that *o*-cymen-5-ol MIC values were in the range 1.66 mM and 3.33 mM and MBC between 3.33 mM and 6.66 mM; for zinc gluconate the MIC was between 2.8 mM and 11 mM and MBC between 11.00 mM and > 44.02 mM. Combinations of the two agents in solution showed significant synergistic effects (FICI \leq 0.50) in MIC and MBC tests against oral anaerobic species, with effective MIC values as low as 0.42 mM/0.69 mM for the combination of *o*-cymen-5-ol/zinc gluconate, respectively.

Antibacterial activity of solutions and whole toothpastes

Solution data are presented for anaerobic species in Figures 1 and 2. Significantly increased antimicrobial activity was seen against *P. gingivalis* and *F. nucleatum* for the combination of the three agents than for the individual agents alone. Presumably because of interactions with SLS and flavours, whole toothpastes gave no recoverable counts for these organisms.

Data comparing the EN1276 kill times versus *E. coli* and *S. mutans* for 16.67% w/w dilutions (1/6) of

Table 1 MIC, MBC & Synergy of *o*-cymen-5-ol & zinc gluconate

Bacterial Species & Strain	Individual MIC (mM)		Combination MIC (mM)	FICI [§]	Synergy [†]
	<i>o</i> -Cymen-5-ol	Zinc gluconate			
<i>S. mutans</i> NCTC 10449	1.66	2.76	0.83/1.38	1.00	No
<i>A. viscosus</i> NCTC 10951	3.33	11.03	1.66/5.51	1.00	No
<i>F. nucleatum</i> NCTC 10562	3.33	5.51	0.83/1.38	0.50	Yes
<i>P. gingivalis</i> ATCC 53978	1.66	2.76	0.42/0.69	0.50	Yes
<i>C. albicans</i> NCPF 3179	1.66	5.51	ND	–	–
Bacterial Species & Strain	Individual MBC (mM)		Combination MBC (mM)	FICI [§]	Synergy [†]
	<i>o</i> -Cymen-5-ol	Zinc gluconate			
<i>S. mutans</i> NCTC 10449	3.33	11.03	1.66/5.51	1.00	No
<i>A. viscosus</i> NCTC 10951	6.66	11.03	3.33/5.51	1.00	No
<i>F. nucleatum</i> NCTC 10562	3.33	22.05	1.66/1.38	0.56	No
<i>P. gingivalis</i> ATCC 53978	3.33	11.03	0.83/2.76	0.50	Yes
<i>C. albicans</i> NCPF 3179	3.33	>44.11	ND	–	–

* for ease of reference concentrations in toothpaste active system are *o*-cymen-5-ol = 6.66 mM; zinc gluconate (added as zinc chloride) equivalent to 44.11 mM.

[§] FICI = MIC_{*o*-Cymen-5-ol+Zinc gluconate}/MIC_{*o*-Cymen-5-ol alone} + MIC_{Zinc gluconate+*o*-Cymen-5-ol}/MIC_{Zinc gluconate alone}

[†] Synergy defined as FICI \leq 0.50^{31,52}.

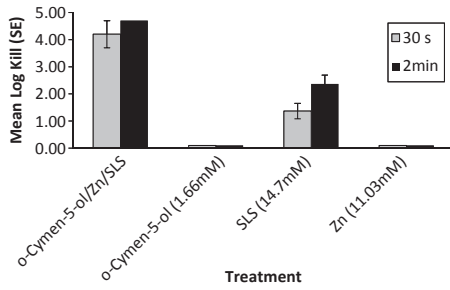


Figure 1. *F. nucleatum* Solution Kill Time ($n = 3$).

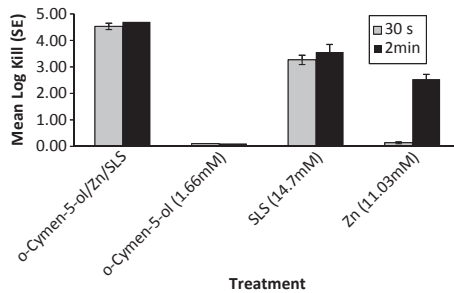


Figure 2. *P. gingivalis* Solution Kill Time ($n = 3$).

toothpastes are presented in Figures 3 and 4 for two independent experiments on toothpastes. The data were compared by ANOVA with factors for treatment and experimental run. The combination of *o*-cymen-5-ol and zinc chloride in toothpaste showed greater effects

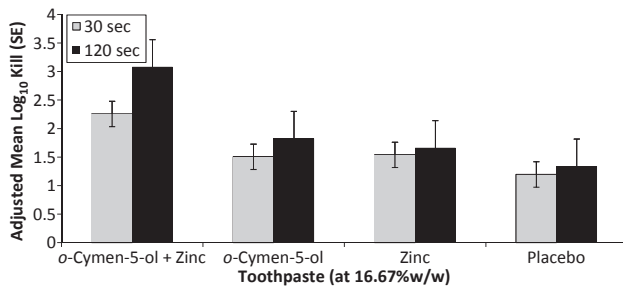


Figure 3. *E. coli* Toothpaste Kill-Time ($n = 6$).

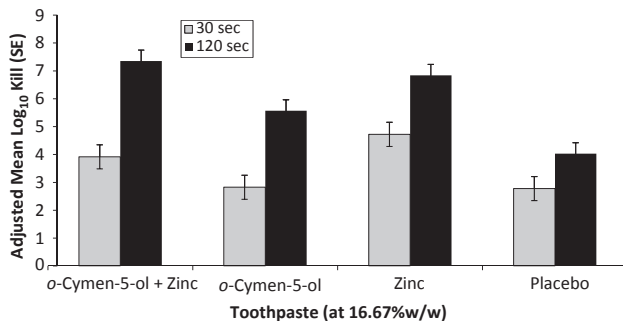


Figure 4. *S. mutans* Toothpaste Kill-Time.

against both *S. mutans* and *E. coli* than placebo pastes at both time points. This difference was significant ($P < 0.0001$) for *S. mutans* at 120s (\log_{10} kill = 7.35 ± 0.40 versus \log_{10} kill = 4.02 ± 0.40), and significant at both 30s ($P = 0.0032$) and 120s ($P = 0.0183$) for *E. coli*. Zinc only paste showed directionally greater kill than the combination paste at the 30s time point against *S. mutans*, whereas the combination paste showed directionally better kill at 120s. Data for 25% dilutions showed similar trends, but were less differentiated since the kill was closer to the maximum kill detectable in the test for all pastes.

Glycolysis inhibition

Data on glycolysis inhibition are presented in Figure 5. The agents tested were found to act mainly additively on acid production. For example, the data presented in Figure 5 show that *o*-cymen-5-ol and $ZnCl_2$ were both inhibitory and, in combination, had additive action. Other combinations of the antimicrobials could have dramatic effects. For example, either 20 mM *o*-cymen-5-ol or 0.2 mM SLS reduced glycolytic acid production by some 50%; together they totally inhibited the glycolytic process (data not shown).

Protease inhibition

Protease was inhibited in a dose dependent fashion by zinc gluconate, with >50% inhibition of activity at less than 1 mM zinc gluconate (Figure 6). *o*-Cymen-5-ol showed no inhibitory effect on *P. gingivalis* protease activity (data not shown).

DISCUSSION AND CONCLUSIONS

The data presented above describe the wide ranging activity of the key components of the novel system containing *o*-cymen-5-ol and zinc. The MIC data

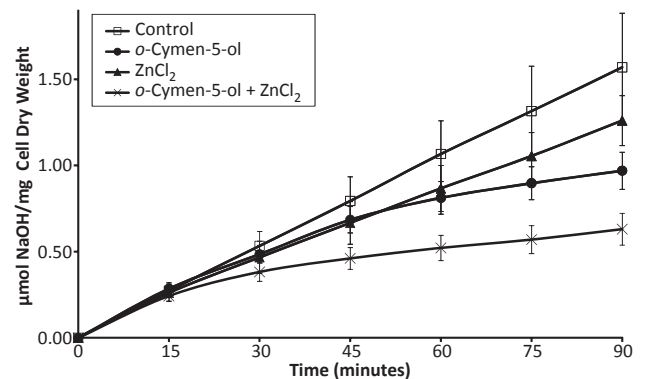


Figure 5. Inhibition by 10mM *o*-Cymen-5-ol & 1 mM Zinc Chloride of *S. mutans* UA159 Glycolysis (pH 6.0; 1.1 mg/ml dry weight; $n = 6$). (Error bars = S.E.)

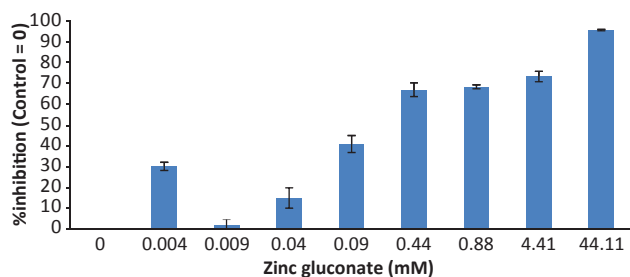


Figure 6. Zinc Inhibition of *P. gingivalis* Protease. (Error bars = S.E.)

demonstrate a broad range of activity for inhibition of growth against a representative selection of typical oral micro-organisms. The MIC values all lie in the range between 1/4 and 1/16 of the concentrations of the antimicrobial system in toothpaste, with zinc values generally relatively lower (as a fraction of toothpaste concentrations) than those for *o*-cymen-5-ol.

Effects of additional antimicrobial agents in toothpaste formulations are often difficult to discern because other formulation components, especially surfactants and to a lesser degree flavour components, also deliver significant effects. In short contact kill time assays of 1/6 toothpaste slurries the effects of the *o*-cymen-5-ol/zinc chloride system are clear against both *S. mutans* and *E. coli* (Figures 3 and 4), in comparison to placebo formulations containing an identical formulation without the system. The combination of agents was superior to the placebo containing neither active, enhancing the antimicrobial effects of the base placebo paste significantly at $P < 0.05$ for all conditions tested except for *S. mutans* at 30s, where it was directionally superior. *E. coli* was included as a comparator in these tests since although it is not directly relevant in the oral cavity, its relative insensitivity to SLS and flavour effects mean it is a useful marker organism for testing antimicrobial additives in whole toothpaste formulations. In the case of *S. mutans* the differential effect was largely driven by zinc; for *E. coli* the effects of *o*-cymen-5-ol were greater. Although the antimicrobial effects of SLS tend to be relatively large in short-contact tests, the significance of SLS effects *in vivo* are likely to be limited by the relatively short residence time of this agent in the oral cavity⁵⁵. In contrast, data for zinc show lengthy residence times in saliva and longer still in plaque⁵⁶, sometimes several hours. There are no direct data currently available for *o*-cymen-5-ol.

The effects of antimicrobial agents at such low concentrations are of great interest, since these are likely to be significant in delivering benefits that span the time periods between tooth brushing events⁵⁷. Synergy is often discussed in evaluation of combination antimicrobial systems, but is often poorly defined⁵¹. We have applied a stringent definition, where the fractional inhibitory concentration index (FICI) must be ≤ 0.50 to

indicate synergy^{51,52}. This allows for variation in doubling dilution MIC/MBC determination, as well as excluding definitions of merely 'additive' effects included in some publications. By this definition, data for the combination of zinc and *o*-cymen-5-ol show synergy against the anaerobes *F. nucleatum* and *P. gingivalis* (4-fold differences in combined MIC compared with MIC for single agent), with no evidence of synergy against *S. mutans* or *A. viscosus*. Similarly, FICI for both anaerobes in MBC was lower than for either *S. mutans* or *A. viscosus*, although only against *P. gingivalis* was there evidence of synergy.

Anti-metabolic effects are also likely to be significant in determining the effectiveness of agents in oral care products. Such effects can act both to slow the (re)growth of plaque bacteria after oral care routines, thus reducing the plaque microbial challenge. They can also reduce the production of metabolites which in themselves are deleterious to host tissues. Data presented here shows both glycolytic and proteolytic enzymes can be retarded by one or both of the two agents studied.

Inhibition of the arg-gingipain activity of *P. gingivalis* extended across a wide concentration range for zinc gluconate, to sub-millimolar concentrations; no significant inhibition by *o*-cymen-5-ol was observed. This inhibitory effect has been reported briefly previously³⁷. Such sub-MIC effects of antimicrobial agents have been suggested to be an important property of antimicrobial agents in oral care usage⁵⁸, because they extend the spectrum of potential clinical effect over much greater lengths of time, in some cases potentially extending this as far as the next tooth brushing. At the same time, sub-MIC effects are likely to avoid deleterious effects of more overt antimicrobial action.

Inhibition of glycolytic acid production would reduce the cariogenicity of mutans streptococci and other related organisms involved in caries, and both agents show effects, with an additive effect of the combination. In addition to the data presented here, Burnett *et al.*⁴⁹ have shown in both simple and complex microbial model systems that the agents discussed can reduce VSC production.

Taken together, the overt antimicrobial data on simple solutions and toothpastes, together with the data indicating synergistic MIC/MBC effects, as well as inhibition of key bacterial metabolic processes associated with oral diseases suggest a potential antimicrobial basis for the clinical efficacy observed in clinical studies^{59,60}.

CONFLICT OF INTEREST AND SOURCE OF FUNDING

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