Antibacterial agents in the control of supragingival plaque — a review

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Abstract This review considers the main agents which have been used as antibacterial agents in mouthwashes and other vehicles to inhibit the growth of supragingival plaque. The agents discussed are bisguanide antiseptics, quaternary ammonium compounds, phenolic antiseptics, hexetidine, povidone iodine, triclosan, delmopinol, salifluor, metal ions, sanguinarine, propolis and oxygenating agents. The plaque inhibitory, anti-plaque and anti-gingivitis properties of these agents are considered along with their substantivity, safety and possible clinical usefulness. Clinical trials of these agents that have been published are also reported. The possible clinical uses of antiseptic mouthwashes are finally considered along with some advice about assessing manufacturers claims. Throughout this review the terms plaque inhibitory, anti-plaque and anti-gingivitis have been used according to the clarification of terminology suggested by the European Federation of Periodontology at its second workshop. This defines a plaque inhibitory effect as one reducing plaque to levels insufficient to prevent the development of gingivitis; an anti-plaque effect as one which produces a prolonged and profound reduction in plaque sufficient to prevent the development of gingivitis; and anti-gingivitis as an anti-infammatory effect on the gingival health not necessarily mediated through an effect on plaque.

Antimicrobial and plaque inhibitory agents in mouthwashes or toothpastes, used to inhibit bacterial plaque formation and thus to prevent or resolve chronic gingivitis, can only affect supragingival plaque. They should be clearly distinguished from agents directed against subgingival plaque which may be used to treat chronic periodontitis and which need to gain access to the periodontal pocket in sufficient concentration to produce their effect.

Supragingival plaque control

A number of antimicrobial agents have been studied in respect to the control of supragingival plaque and they can be divided into bisguanide antiseptics, quaternary ammonium antiseptics, phenolic antiseptics, other antiseptics, oxygenating agents, metal ions and natural products.[1]

Bisguanide antiseptics

Several bisguanide antiseptics possess anti-plaque activity, including chlorhexidine, alexidine and octenidine. Chlorhexidine gluconate, however, is the most studied bisguanide and is the one on which there is most information on toxicology.[1]

Bisguanide antiseptics are able to kill a wide range of microorganisms by damaging the cell wall. The anti-plaque properties of chlorhexidine are unsurpassed by other agents and it has much greater and more prolonged effects than other antiseptics of similar or greater antibacterial activity. This appears to be caused by the adsorption of the dicationic chlorhexidine molecule onto oral surfaces and its release at bacteriocidal levels over prolonged periods.

Chlorhexidine

The digluconate of chlorhexidine (1:6-Di 4'-chlorophenyl-diguanidohexane) is a synthetic antimicrobial drug which has been widely used as a broad spectrum antiseptic in clinical and vetinary medicine since 1953. It has been available in Europe for more than 25 years and has been successfully used in the dental field during that period.

As an antimicrobrial agent, chlorhexidine is effective *in vitro* against both Gram-positive and Gram-negative bacteria[2–4] including aerobes and anaerobes[2] and yeasts and fungi.[5] Its antibacterial action is due to an increase in cellular membrane permeability followed by coagulation of the cytoplasmic macromolecules.[6] It has also been shown that chlorhexidine can reduce the adherence of *Porphyromonas gingivalis* to epithelial cells.[7] This effect is probably due to the binding of chlorhexidine to the bacterial outer membrane and therefore it could have similar results on the adherence of other plaque bacteria.

It has been shown that a 0.2% chlorhexidine gluconate mouthrinse will prevent the development of experimental gingivitis after the withdrawal of oral hygiene procedures.[1,8] It has thus been shown to be both a highly effective anti-plaque agent. However, when used as an adjunct to normal oral hygiene measures, variable results are achieved, suggesting that chlorhexidine is more effective in preventing plaque accumulation on a clean tooth surface than in reducing pre-existing plaque deposits.

Chlorhexidine is thus able to inhibit plaque formation in a clean mouth but will not significantly reduce plaque in an untreated mouth. For these reasons chlorhexidine mouthwash should never be given to patients before the necessary periodontal treatment has been carried out and then should only be used for the specific reasons set out below.

Substantivity of chlorhexidine

The ability of drugs to adsorb onto and bind to soft and hard tissues is known as substantivity and this property was first described for chlorhexidine in the 1970s.[9–11] Substantivity is influenced by the concentration of the medication, its pH and temperature, and the length of time of contact of the solution with the oral structures.[10] This property of chlorhexidine was associated with its ability to maintain effective concentrations for prolonged periods of time[11,12] and this prolongation of its action made it especially suitable for the inhibition of plaque formation.

Safety of chlorhexidine

The safety of an antimicrobial agent should be tested in animal studies prior to its clinical use. Any side effects found are then carefully investigated in human studies. The effects of their metabolic products on the environment are also frequently studied.

Animal experiments with radiolabelled chlorhexidine have shown that the primary route of excretion is through the faeces. There is minimal metabolic cleavage and there has been no reported

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evidence of carcinogenic substance formation.[13] Chlorhexidine is poorly absorbed by the gastrointestinal tract and it therefore displays very low toxicity (oral LD50 is 1800 mg/kg and the intravenous LD_{50} is 22 mg/kg). No tetragenic alterations have been found following long-term use.[14]

The most common side effect of chlorhexidine is the formation of extrinsic stain on the teeth and tongue following its use as a mouthwash.[1]

Clinical usage (fig.1)
There are now quite a few commercially available chlorhexidine mouthwashes in the UK and the rest of Europe. Those in the UK, such as Corsodyl, contain 0.2% chlorhexidine and recommend a 10 ml volume per rinse. The chlorhexidine mouthwash available in the USA, Peridex, contains 0.12% chlorhexidine and recommends a 15 ml volume per rinse. The factor governing the effectiveness of these mouthwashes is the total dose of chlorhexidine delivered and 10 ml of 0.2% solution delivers 20 mg and 15 ml of 0.12% solution delivers 18 mg.[15] Since both of these amounts are similar and above the therapeutic dose, either of the formulations is equally effective.

Both chlorhexidine and fluoride may have valuable preventive roles in dental disease and there is also evidence that in caries prevention they may act together to provide additional benefits. For this reason combined chlorhexidine and fluoride have been investigated. One study used a 0.12% chlorhexidine and 100 ppm F^- mouthrinse in combination with toothbrushing in a randomised, double blind parallel design involving 99 subjects over 6 weeks. At 6 weeks the plaque and gingivitis scores were significantly lower in the active mouthrinse group. As expected some tooth staining also occurred. The anti-plaque effects were the same as with a conventional chlorhexidine mouthwash. Similar results were seen in a study using a 0.05% sodium fluoride and 0.05% chlorhexidine mouthwash.[17]

It is more difficult to incorporate chlorhexidine into toothpastes and gels because of the binding of chlorhexidine to components in the toothpaste. This reduces its activity by decreasing the number of active cationic sites.[18] However, some formulations have been achieved which avoid this problem. In comparing the effect of potential anti-plaque ingredients in toothpastes, the plaque inhibitory effects of the other ingredients need to be taken into account. In this regard, it has been shown that commercial toothpastes containing various formulations of fluoride all reduce the rate of plaque regrowth compared with water in a 4-day study.[19]

More recently, some toothpastes have been specifically formulated to ensure a high availability of the contained antiseptic. A 1% chlorhexidine toothpaste of this type has been investigated in a 19-day, randomised double blind, placebo-controlled, cross-over experimental gingivitis clinical trial.[20] The toothpastes were used as slurries which were rinsed around the mouth twice per day for 1 minute during the experimental period. Plaque and gingivitis scores were highly significantly reduced and stain scores were significantly increased in the active toothpaste period with respect to those in the placebo period. Thus, this particular formulation of chlorhexidine toothpaste does seem to provide a sufficient dose of chlorhexidine for a similar clinical effect to that seen with chlorhexidine mouthrinsing.

Chlorhexidine has also been incorporated into a sugar-free chewing gum (Fertin A/S, Vejle, Denmark), and in this form the chlorhexidine molecule remains unbound. The chewing gum contains 20 mg of chlorhexidine diacetate and this has been compared with the effects of a 0.2% chlorhexidine mouthwash and a placebo gum in a clinical study.[21] The 151 subjects were divided into three groups, one using the chlorhexidine gum, one 0.2% chlorhexidine mouthwash and one a placebo gum. These groups were tested for their anti-plaque effects after 4 and 8 weeks. The subjects using the gum chewed two pieces twice per day for 10 minutes and the mouthwash subjects rinsed twice per day for 1 minute. There were significant and similar anti-plaque effects from the use of the chlorhexidine gum and mouthwash, and these were not seen with the placebo gum. Tooth staining was seen both with the chlorhexidine gum and mouthwash but the intensity and extent of stain was less with the gum.

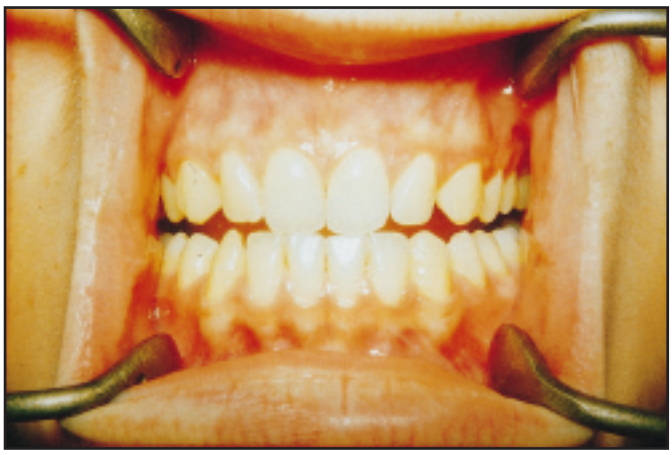

Fig. 1 A healthy mouth in a subject with excellent oral hygiene. The teeth are free of visible plaque. It would be possible to maintain this healthy periodontal state by the use of an effective anti-plaque mouthwash such as chlorhexidine if this subject were unable to brush their teeth for a short period for any reason

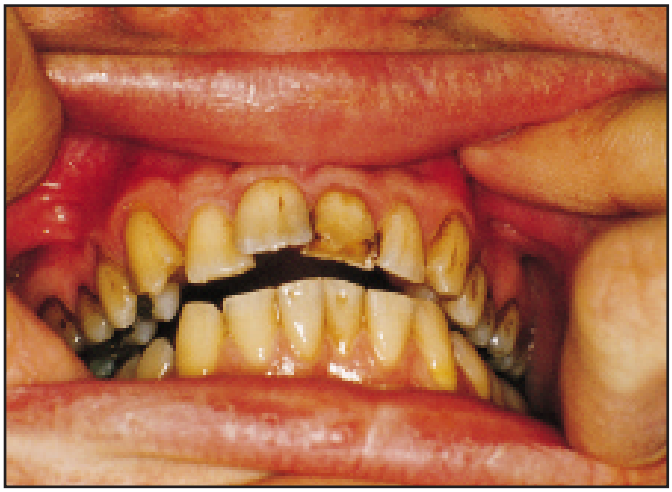

Fig. 2 Brown staining of the teeth resulting from the use of a 0.2% chlorhexidine gluconate mouthwash over a 4-week period

In a similar study, the use of chlorhexidine gum has also been found to reduce plaque levels significantly more than the use of xylitol and sorbitol gums.[22] Therefore, the use of chlorhexidine gum could be a good method of using chlorhexidine in longer term users.

Side effects of chlorhexidine usage (fig. 2)
Although chlorhexidine is not toxic, it has an unpleasant taste, alters taste sensation and produces brown staining on the teeth which is very difficult to remove. This can also affect the mucous membranes and the tongue and may be related to the precipitation of chromogenic dietary factors on to the teeth and mucous membranes. It is probable that one cationic group attaches chlorhexidine to the tooth or mucosal surface, while the other cationic group produces the bactericidal effect of damaging the bacterial cell wall. However, this cationic group can also attach dietary factors such as gallic acid derivatives (polyphenols) found in some foods and many beverages including tea and coffee and tannins from wines to the molecule and hence to the tooth surface.[23] Chlorhexidine also encourages

supragingival calculus formation[24] and the resulting calcified and stained areas adhere strongly to the tooth (or restoration) surface and are difficult to remove.

The stained areas are resistant to polishing and can only be removed by scaling and in this regard ultrasonic scaling is the most effective method. They also tend to stain the margins and surfaces of composite and glass-ionomer restorations, and these stains are particularly resistant to removal by scaling. Scaling procedures are also liable to damage the surface of these restorations and therefore reduce their effective life.

It is important for these reasons to advise patients using chlorhexidine mouthwash to avoid the intake of tea, coffee and red wine for the duration of its use. One should also severely restrict its use in patients with visible anterior composite and glass-ionomer restorations.

It is also worth stating that chlorhexidine formulations which do not stain are ineffective in inhibiting plaque. This is because the second cationic group of the molecule has reacted with something in the formulation making it unavailable for either a beneficial bactericidal effect or the unwanted staining effect. This has been shown clearly in a comparison of a number of commercial chlorhexididine mouthwashes which differed in their content of binding additives. Those which effectively bound up the chlorhexidine did not produce staining but also lacked an anti-plaque effect.[25,26] Mouthwashes with this reduced effect include French Eludril. The formula of British Eludril has now been changed to prevent the binding of chlorhexidine. As a result this product is now effective and causes tooth staining similar to the other effective products.

In an effort to reduce staining, anti-adhesive molecules have been combined with chlorhexidine in experimental mouthwashes. These combined mouthwashes have no effect on 4-day plaque regrowth, and do not cause increased tea staining almost certainly for the same reasons stated above.[27,28]

Other much rarer side effects of chlorhexidine mouthwash are mucosal erosion and parotid swelling.[1]

For these reasons, the prolonged use of chlorhexidine should be avoided in normal periodontal patients. It is useful for short periods of up to 2 weeks when oral hygiene may be difficult or impossible, such as during acute oral infections or following periodontal or other forms of oral surgery. It may occasionally be used as an adjunct to mechanical oral hygiene in initial periodontal treatment. In this regard, the gingivae may be sore after subgingival scaling and this may prevent normal toothbrushing. However, this effect does not usually last more than 2 to 3 days and therefore the use of the mouthwash is not usually necessary for more than this period. At this point normal brushing and flossing must be resumed and mouthwashing should stop.

Chlorhexidine mouthwash may also be used during periods of intermaxillary fixation following the treatment of fractures or skeletal surgery when effective oral hygiene is not possible lingually and interdentally. During this period patients should also be seen regularly for professional cleaning by a dentist or hygienist to limit staining.

More prolonged use of chlorhexidine may be justified in physically and mentally handicapped patients, in medically-compromised patients predisposed to oral infections and as an adjunct to oral hygiene in fixed orthodontic appliance wearers. All of these patients should also be seen for regular professional cleaning. In many of these special cases the mouthwash or gel will be used over a prolonged period and severe staining will be a problem. This can be minimised by using concomitant toothbrushing and by avoiding the intake of certain foods and drinks such as tea and coffee (see earlier).

With increasing pocket depth subgingival plaque becomes inaccessible to both oral hygiene procedures and antibacterial mouthrinses which are normally effective in preventing gingivitis. In this regard it has been shown that mouthwashes do not penetrate into the gingival crevice or periodontal pocket.[29,30] Therefore, antibacterial mouthrinses, toothpastes and gum have no place in the treatment or control of periodontitis.

Quaternary ammonium compounds

Quaternary ammonium compounds such as cetylpyridinium chloride (CPC) have moderate plaque inhibitory activity.[31,32] Although they have greater initial oral retention and equivalent antibacterial activity to chlorhexidine, they are less effective in inhibiting plaque and preventing gingivitis. One reason for this may be that these compounds are rapidly desorbed from the oral mucosa.[11,33,34] It has also been found that the antibacterial properties of these compounds are considerably reduced once adsorbed onto a surface and this may be related to the monocationic nature of these compounds. The cationic groups of each molecule bind to receptors on the mucosa producing the mucosal retention but because of the monocationic nature of these molecules this process leaves few unattached sites available for its antibacterial function.

A CPC pre-brushing mouthrinse used as an adjunct to mechanical oral hygiene has not been found to have an additional beneficial effect on plaque accumulation.[35] With regard to conventional use, one study compared the plaque-inhibitory potential of 0.05% and 0.1% CPC, 0.05% chlorhexidine and control mouthrinses used twice daily during a 4-day period of non-brushing.[36] The 0.1% CPC-rinse had the lowest plaque scores, being around 26% lower than the control rinse, and 7% lower than the 0.05% chlorhexidine rinse. The 0.05% CPC and chlorhexidine mouthwashes were very similar in their effects. The relatively poor effect of the 0.05% chlorhexidine and CPC mouthwashes is undoubtedly due to the low concentration in these formulations yielding too low a total dose for the expected effect. Also, the short duration of this study makes it impossible to detect any effect on gingivitis which would be expected from a normal chlorhexidine mouthrinse. It does, however, show that the CPC 0.1% mouthwash did produce a limited but statistically-significant reduction in plaque growth.

A slow release system containing CPC has been tried to increase the retention time for CPC in the mouth.[37] The plaque inhibitory effect over 18 days of this device was compared with that of a CPC mouthrinse, CPC lozenges (Cepacol) and a chlorhexidine mouthrinse (Peridex). As expected, the chlorhexidine mouthrinse (Peridex) had the most profound effects and these were not approached by the other formulations. However, there were no differences between any of the CPC formulations which showed that the slow release system had no effect on the efficacy of CPC. All the CPC formulations and Peridex produced tooth staining and this was worst with the CPC lozenges.

Phenolic antiseptics

Phenols, either alone or in combination, have been used in mouthrinses or lozenges for a considerable time. When used at high concentrations relative to other compounds they have been shown to reduce plaque accumulation.[38–40] Listerine is an essential oil/phenolic mouthwash which has been shown to have moderate plaque inhibitory effects and some anti-gingivitis effects. There have been a number of short and long-term home-use studies which have shown that it has moderate plaque inhibiting effects and some anti-infammatory effects in reducing gingival inflammation.[41–43] On the basis of these studies it has been accepted by the American Dental Association to be an aid to home oral hygiene measures.

Its effects on 4-day plaque regrowth during abstinence from mechanical oral hygiene has been compared with those from chlorhexidine and anti-adhesive mouthwashes.[27] 0.2% chlorhexidine mouthrinse was significantly more effective than Listerine which was in turn more effective than the anti-adhesive mouthwashes alone

or in combination with chlorhexidine,which it inactivates (see earlier). It was, however found to be slightly more effective than triclosan mouthwash in plaque inhibition.[44] Its anti-inflammatory effects shown in the home use studies may be due to its antioxidative activity.[45] Thus, Listerine has a moderate effect on plaque regrowth and some anti-inflammatory effect which may reduce the severity of gingivitis. Its lack of profound plaque inhibitory effect is probably because, unlike chlorhexidine, it has poor oral retention.

Hexetidine

Hexetidine has some plaque inhibitory activity but this is low in comparison with chlorhexidine.[25,26,34,46] Its substantivity (oral retention) is between 1 and 3 hours,[26] which accounts for the reported low plaque inhibitory effects of Oraldene, the UK product.[34] Another study investigated its effect on the healing of aphthous ulcers and did not show any added benefit over mechanical oral hygiene alone.[47] Moreover, this agent at concentrations greater than 0.1% can cause oral ulceration.[46] It has also been shown that combining zinc with hexetidine improves its plaque inhibiting activities probably by acting synergistically with it.[48]

Povidone iodine

Povidone iodine appears to have no significant plaque inhibitory activity when used as 1% mouthwash[49] and the absorption of significant levels of iodine through the oral mucosa may make this compound unsatisfactory for prolonged use in the oral cavity.[50] Also, it could cause a problem of iodine sensitivity in sensitised individuals.

Triclosan

Triclosan, a trichlora-2'-hydroxydiphenyl ether, is a non-ionic antiseptic which lacks the staining effects of cationic agents. It has been used recently in a number of the commercial toothpastes and mouthwashes, and produces moderate plaque inhibitory effects when used as a mouthwash in combination with zinc.[51,52] In one study,[51] the combination mouthwash produced inhibition of plaque regrowth during a 4-day period with abstinence from mechanical oral hygiene but this study raised doubts as to the individual contribution of triclosan to this effect . The use of a combination of zinc and triclosan arose from the concept that agents with different modes of action might have synergistic or additive effects. However, the separate and combined effects of triclosan have been investigated and these are described later.

Fig 3 A patient carrying out mechanical toothbrushing. It is possible that the increased effect on the bacterial plaque might be achieved if this procedure were supplemented by a pre-brush rinse with an antibacterial mouthwash. It would, however, be essential that this agent did not producing staining. Such an effect might also be produced by the use of a toothpaste containing such an agent eg triclosan along with copolymer to increase its oral retention time

The effects of combination zinc and triclosan mouthwashes were investigated in a 3-week clinical trial,[52] where abstinence from brushing was produced by wearing an acrylic tooth shield over the test area of the mouth during brushing. Two experimental mouthwashes containing 0.4% zinc sulphate and 0.15% triclosan were compared with a 0.12% chlorhexidine mouthwash and a placebo (negative control) mouthwash. The two experimental mouthwashes differed only in their ethanol and humectant content. The mouthwashes were used twice daily after brushing for 3 weeks. In the negative control subjects the plaque and gingival bleeding scores rose above their pre-study levels. In the subjects using the first zinc/triclosan mouthwash the plaque levels were significantly lower than the control levels. However, this difference was not significant for the second zinc/triclosan mouthwash. The first zinc/triclosan mouthwash had higher concentrations of ethanol and humectant which probably improved the effect by increasing the solubilisation of triclosan which has a low solubility in water. As expected the plaque and gingivitis scores were the lowest in the subjects using chlorhexidine mouthwash.

The effects of these same two experimental zinc/triclosan mouthwashes were also compared with a non-active control mouthwash over 28 weeks.[53] The subjects were divided into three groups and each was given one of the three mouthwashes which they used twice per day after brushing. Assessments were made of the clinical status and levels of salivary *Streptococcus mutans* . At 4 weeks, plaque and calculus scores were low compared to baseline for all the groups but thereafter they progressively increased. Plaque and gingival bleeding scores were significantly lower in subjects using the experimental mouthwashes than those using the control mouthwash. Calculus scores were also significantly lower at 28 weeks for the subjects using the second experimental mouthwash. No significant changes in salivary *Streptococcus mutans* numbers were seen. The only adverse effect seen was some tooth staining.

Another crossover study compared the effect of 0.06% triclosan, 0.12% chlorhexidine and placebo mouthwashes on *de novo* plaque formation over 18 days at healthy and inflamed gingival sites of ten volunteers.[54] No significant differences in the gingivitis scores were found between the three mouthwashes but both active mouthwashes produced significant reductions of plaque formation compared to the control mouthwash. These reductions were significantly greater for the chlorhexidine compared with the triclosan mouthwash. They also found that more plaque formed at inflamed sites than healthy sites regardless of which mouthwash was used.

While triclosan itself has little or no substantivity there is evidence that its oral retention can be increased by its combination with copolymers of methoxyethylene and maleic acid (Gantrex, ISP Corp).[55,56] Furthermore, there is also evidence from two short-term trials[55,56] and two longer-term trials[57,58] conforming to American Dental Association Guidelines[59] that the combination of 0.03% triclosan with Gantrex used as a pre-brushing rinse can produce significant adjunctive effects to mechanical oral hygiene in further reducing plaque and gingivitis levels.

Moreover, there is evidence that triclosan may also act as an anti-inflammatory agent in mouthrinses and toothpastes.[60] In this way it has been shown to reduce the inflammatory reaction produced on the gingiva[61] and skin[62] by sodium lauryl sulphate, and the skin reaction to nickel hypersensitivity.[63] In addition, it has been shown to reduce histamine-induced dermal inflammation and reduce the severity and healing period of aphthous ulceration.[64] The mechanism of this property has been investigated *in vitro* [65] and triclosan has been shown to inhibit both cyclo-oxygenase and lipoxygenase,

and thus reduce the synthesis of prostaglandins and leukotrienes which are key mediators in the inflammatory reaction.

This issue is further complicated by the fact that the anti-inflammatory and anti-bacterial properties of triclosan combinations are affected by the nature of the solvents in the formulation.[66–70]

Thus, triclosan mouthwashes reduce plaque accumulation but to a much lesser extent than chlorhexidine. However, the extent of their plaque inhibitory effect seems to be dependent upon the presence of co-polymers in the formulation to increase oral retention of triclosan. Any effects of triclosan on gingivitis levels are probably due to its anti-inflammatory effect. The anti-inflammatory effect of triclosan also depends upon its ability to penetrate into the gingival tissues and this is in turn dependent upon the nature of the solvent(s) in the mouthwash formulation.

Triclosan has also been added to a number of experimental and commercial toothpastes with and without zinc and these appear to produce moderate inhibition of plaque formation.[71,72] These and other studies have shown that zinc citrate and triclosan toothpastes,[71–78] and triclosan/copolymer toothpastes[79–81] have produced greater reductions of plaque and gingivitis than brushing alone (fig. 3). However, one study has shown that it has plaque inhibitory effects which are little different from other detergent-based commercial toothpastes regardless if it is present with or without zinc.[82]

The effects of a triclosan dentifrice on the microbial composition of supragingival plaque over 6 months has also been studied.[83] Of 144 subjects, these were divided into two groups and one was given 0.3% triclosan/0.2% copolymer/0.243% sodium fluoride toothpaste and the other a placebo toothpaste. Both test and placebo dentifrices produced significant reductions in the total bacterial counts and a non-significant reduction in the anaerobic count. Neither dentifrice resulted in detrimental shifts in the microbial composition of the flora nor to the emergence of periodontal or opportunistic pathogens. There was also no difference in the proportion of the flora resistant to triclosan regardless of whether the triclosan or placebo toothpaste was used. Thus, the extended use of a 0.3% triclosan/0.2% copolymer toothpaste appears to be safe to use and does not seem to disrupt the normal oral flora.

In another study,[84] the effects of three commercial triclosan toothpastes, Colgate Paradent (triclosan/copolymer), Pepsodent Gum Health (triclosan/zinc citrate), Dentosal Friskt Tandkött (triclosan/pyrophosphate), and a placebo toothpaste on plaque, gingivitis and the salivary microflora were compared over 6 months in 112 subjects. By the end of the 6-month study Colgate Paradont reduced plaque scores by 36%, and Pepsodent Gum Health by 6%; there were increased scores of 5% for Dentosal Friskt Tandkött and 2% for the placebo. Gingival bleeding scores reduced in all groups with no significant differences between them. There was an increase in the number of streptococci over time with Dentosal, Pepsodent and placebo, but not Colgate toothpaste. This would seem to indicate that only the triclosan/copolymer formulation significantly reduced plaque levels with respect to the control during this period of normal use.

Another study also compared the effects of a commercially available triclosan/copolymer toothpaste, a sodium fluoride containing toothpaste, a chlorhexidine rinse (positive control) and saline rinse (negative control) on 4-day plaque regrowth.[15] The toothpastes were made into slurries for rinsing around the mouth so that the compounding effect of mechanical brushing was avoided. Eighteen health volunteers took part in the study. On day 1, they suspended toothbrushing and rinsed twice daily with the allocated mouthrinse or toothpaste slurry. Chlorhexidine was significantly more effective than all the other agents tested, and both toothpastes were significantly better than the saline rinse. There was no significant difference between the two toothpaste rinses.

These studies show that triclosan toothpaste offers only moderate plaque inhibiting properties when compared with conventional toothpaste. However, they have also been shown to reduce gingival inflammation further than mechanical brushing alone when used as an adjuvent to normal brushing[70–81] and this may be associated with triclosan's anti-inflammatory properties. However, these effects are much less profound when triclosan toothpastes are used as slurries to mitigate against the confounding effects of mechanical plaque removal, and in this form they are no more effective than a conventional toothpaste without triclosan or any other antimicrobial agent.[15,82]

Delmopinol

Several substituted amine alcohols such as octapinol hydrochloride have been shown to inhibit plaque accumulation.[85,86] More recently studies have been carried out on the related morpholinoethanol derivative, delmopinol hydrochloride. Both *in vitro*[87] and *in vivo* studies[88] show that it inhibits plaque growth and reduces gingivitis. One study has suggested that delmopinol has only limited substantivity in comparison with chlorhexidine and in this regard inhibited salivary bacteria for only 30 minutes as compared to several hours for chlorhexidine.[89] However, the substantivity test used in this study was designed for antibacterial agents that act directly on bacteria and thus reduce their numbers. Since delmopinol is not a true anti-bacterial agent in this sense and has virtually no inhibitory concentration it is not correct to test its substantivity in this way.

A suggested mode of action for its plaque inhibiting effects is interference with plaque matrix formation and reduction of bacterial adherence.[90] This would cause the plaque to be more loosely adherent to the tooth so that it would be more easily removed by mechanical cleaning procedures, and would therefore be suitable for a pre-brush mouthrinse.

A trial of 0.1% and 0.2% delmopinol hydrochloride mouthrinses as adjuncts to normal oral hygiene has been carried out.[91] This investigation was a 6-month home-use, placebo-controlled, double-blind, randomised parallel design study and was structured to conform with the ADA Council of Dental Therapeutics guidelines. 450 healthy subjects with moderate levels of plaque and gingivitis were recruited and were either given one of the delmopinol mouthrinses or a placebo mouthrinse to use twice a day after brushing. They were scored for plaque, gingivitis, tooth stain and supragingival calculus at baseline, and 3 and 6 months. Plaque was also collected for microbiological analysis. In addition, the oral mucosal was examined and they were questioned about adverse reactions. Finally, at the start and end of the trial a full medical examination, including haematological and biochemical tests, was carried out. A few adverse signs and symptoms were reported and these included transitory numbness of the tongue, tooth and tongue staining, taste disturbance and rarely mucosal soreness and erosion. All these local side effects were less commonly reported at 6 months compared to 3 months, and only six subjects withdrew from the study because of adverse events. No systemic effects attributable to the agent were observed and no shifts in haematological and biochemical parameters occurred. All groups showed decreases in plaque, gingivitis and calculus scores with few differences between them but there were some significant differences in plaque scores in favour of 0.2% delmopinol. Tooth staining was increased in the delmopinol groups but not calculus.

The reductions in gingivitis seen in this study suggests that delmopinol may have an anti-inflammatory,[91] and hence an anti-gingivitis effect. In addition, the reductions in both plaque and gingivitis also suggest that it may be a true anti-plaque agent.

The microbiological effects of the above study were investigated on plaque collected at 12, 24 and 36 weeks.[92] There were no consistent effects on the microscopical or total counts. However, there was a significant reduction in the proportion of dextran-producing

streptococci in the active compared with the control group throughout treatment. There was no colonisation by Candida or major shift in bacterial composition in the active group nor was there any decrease in susceptibility to delmopinol. Thus, delmopinol seems to mediate its plaque inhibitory and anti-inflammatory effects without causing a major shift in bacterial populations apart from the reduction in dextran-producing streptococci.

The effectiveness of 0.2 % delmopinol and 0.2% chlorhexidine mouthwashes have also been compared in a 4-week, double blind, randomised, placebo-controlled clinical study of 57 patients with gingivitis.[93] The patients all received professional cleaning before baseline. They were either given delmopinol, chlorhexidine or placebo mouthwashes and told to use 10 ml twice per day after brushing. The plaque index and plaque wet weight were used to score plaque and gingival fluid flow and bleeding on probing to score gingivitis. With respect to plaque, both chlorhexidine and delmopinol significantly reduced scores relative to the placebo and there were no significant differences between the effects of chlorhexidine and delmopinol. However, in respect of gingivitis, there were no significant differences between the effects of delmopinol or placebo mouthrinses This study therefore casts doubt about the the anti-gingivitis effect of delmopinol seen in the study reported earlier.[91] The same adverse effects as described above were reported for both active mouthwashes. A transient anaesthetic effect on the oral mucosa was more commonly reported in the delmopinol group while chlorhexidine produced more tooth and tongue staining than delmopinol.

A recent study,[94] has compared the plaque inhibitory effects of 0.1%, 0.2% delmopinol mouthwashes, and a placebo mouthwash in 'slow' and 'rapid' plaque formers. It confirmed the beneficial effects of both delmopinol mouthwashes versus the placebo but found no differences in their effects on patients with either slow or rapid rates of plaque formation.

Therefore, it would seem that delmopinol is well tolerated and may produce both plaque inhibitory and anti-gingivitis effects. It thus holds promise as a useful agent for mouthwashes and possibly toothpastes.

Salifluor

Salifluor is a salicylanide (5n-octanoyl-3'-trifluoromethylsalicylanide) which has both antibacterial and anti-inflammatory properties.[95] The possibility that 5-alkyl-salicylanides like salifluor may have a plaque-inhibitory effect was suggested by one *in vitro* study.[96] Recently, a combination of salifluor and polyvinylmethylether/ malic acid (OVM/MA) has been investigated *in vitro*[97] and the combination was shown to enhance the uptake of salifluor on saliva-coated hydroxyapatite discs, and to reduce plaque growth in an artificial mouth.

Also recently, three related double blind, randomised, cross-over clinical trials into the effect of mouthrinses containing salifluor on plaque and gingivitis have been carried out.[98] In each study, ten medically and dentally healthy dental students were used and the effects of 0.08%, 0.12% and 0.2% salifluor, 0.12% chlorhexidine and control mouthwashes were compared with a washout period between each. In the first study they found that the salifluor mouthrinses were significantly more effective than the control rinses and equally effective to 0.12% chlorhexidine in retarding 4-day plaque growth.

In the second study, oral hygiene was stopped for 2 weeks to induce gingivitis, and then the teeth were professionally cleaned. Plaque was then allowed to form again for a further 4 days during which time either the control mouthwash, 0.12% salifluor or 0.12% chlorhexidine mouthwash, was used. The results showed that mouthwashes containing 0.12% salifluor and 0.12% chlorhexidine inhibited plaque formation to the same extent at both inflamed and non-inflamed sites, but the effects of both mouthrinses were less effective at inflamed compared with non-inflamed sites.

In the third study, oral hygiene was stopped for 2 weeks and during this time one of the three mouthrinses, 0.12% salifluor, 0.12% chlorhexidine or control mouthwash, was used. Clinical measurements and plaque samples, for dark ground microscopy, were taken at baseline and days 4,7 and 14. The sequence was repeated for each mouthwash with a washout period between each. There was no difference between 0.12% salifluor and 0.12% chlorhexidine mouthwashes in their ability to retard *de novo* plaque formation and the development of gingivitis during the 14-day period. The microbial examination showed that in the control group the percentage of cocci decreased and the percentage of filaments, fusiforms and spirochaetes increased, while in the salifluor and chlorhexidine groups no distinct changes occurred in the composition of the supragingival plaque.

Thus, the results of these three studies show the potential of salifluor as an effective anti-plaque agent. However, the mechanism behind the anti-microbial and anti-inflammatory properties of salifluor are not yet properly understood. Therefore, the clinical use of salifluor should be further studied in the longer term to include a detailed evaluation of possible side effects before it can be released for routine clinical use.

Metal ions

A number of metal ions have been studied for their effects on plaque, and zinc, copper and tin, have been shown to possess plaque inhibitory activity. Both copper and tin suffer from the local side effect of staining. Some fluoride compounds such as stannous fluoride and amine fluorides also have plaque inhibitory activity, but not as a result of the fluoride ion itself but rather due to the effect of the stannous ion or the surface-active amine portion of the molecule.

Studies on the effect of metal ions on plaque accumulation have been contradictory and factors like concentration and frequency of use may explain the differences.[1] However, it has also been shown[99] that zinc is retained by dental plaque and inhibits its regrowth without disrupting the oral ecology. Of further interest is the apparent additive or synergistic effect of the combination of zinc and other metal ions with other antiseptics.[100] This effect has been noted with zinc combined with hexetidine,[48] triclosan,[52] and sanguinarine.[101]

Little is known of the mechanisms by which metal ions exert their effects. It has been suggested that zinc may assist the inhibition of glycolysis by sanguinarine[101] which could in turn limit plaque formation. It has also been reported that they may improve the bactericidal activities of sanguinarine against certain oral organisms and to enhance the efficiency of other antiseptics such as triclosan and hexetidine in inhibiting plaque.

Natural products

Studies on the plant extract sanguinarine chloride have shown that it produces moderate reductions in plaque and gingivitis. The zinc present in the formulations could be partly responsible for the effect.

Sanguinarine

Chemically, sanguinarine is a benzophenanthridine alkaloid derived from the alcoholic extraction of powdered rhizomes of the bloodroot plant, *Sanguinaria canadensis,* that grows in Central and South America and Canada.[102] After precipitation and purification of the alcohol extract, an orange powder containing 30–35% sanguinarine is obtained. Sanguinarine contains the chemically reactive iminium ion which is probably responsible for its activity. It appears to be retained in plaque for several hours after use, and is poorly absorbed from the gastrointestinal tract.[100] Several clinical studies have been carried out into its effects.

A sanguinarine mouthrinse and toothpaste regime given for 6 months during orthodontic treatment reduced plaque by 57%, gingival inflammation by 60%, and bleeding on probing by 45%,

compared with figures of 27%, 21% and 30% for the placebo control group.[103]

A later study of sanguinarine mouthrinse and toothpaste[104] carried out under the ADA guidelines (see earlier) in 120 subjects showed 13–17% lower plaque scores and 16–18% less gingival inflammation compared with a placebo group after a 6-month treatment period.

Reviews on antimicrobial mouthrinses including sanguinarine,[105,106] conclude that short-term studies on sanguinarine have shown variable but significant plaque inhibitory effects but the effect on gingivitis appears to be equivocal. On the other hand, two reviews of sanguinarine toothpastes, used alone without the mouthwash, have shown no detectable plaque inhibitory or anti-inflammatory effects.[107,108]

In respect of its possible modes of action, it has also been shown that sanguinarine at a concentration of 16 μg/ml completely inhibited 98% of microbial isolates from human dental plaque[109] and that sanguinarine and zinc act synergistically in suppressing the growth of various oral strains of streptococci and actinomyces.[110]

Some studies have compared the activity of sanguinarine with other antimicrobial antiseptics. A small group of 14 healthy volunteers were used in an experimental gingivitis study and used either sanguinarine-zinc (Veadent) or chlorhexidine mouthwash.[111] This showed that the chlorhexidine mouthwash was significantly more effective than sanguinarine-zinc in inhibiting plaque formation and the development of gingivitis. The effects of various mouthwashes on 21 patients with gingivitis were examined by another group.[112] Both chlorhexidine and sanguinarine mouthwashes significantly reduced plaque scores compared with a non-active placebo. Another study compared the effectiveness of sanguinarine-zinc (Veadent),[113] chlorhexidine and essential oil/phenolic (Listerine) mouthwashes with a placebo mouthwash in an experimental gingivitis study over 21 days. All these active mouthwashes significantly inhibited plaque accumulation with respect to the placebo, but only chlorhexidine was effective in preventing the development of gingivitis. In a further placebo-controlled study in the USA , the effectiveness of sanguinarine-zinc (Veadent), chlorhexidine and essential oil/phenolic (Listerine) mouthwashes were again compared with a placebo mouthwash, this time in a 6-month study.[114] Again all the active mouthwashes significantly reduced plaque scores compared with the placebo, but only chlorhexidine was able to significantly reduce gingival index and gingival bleeding scores.

There is doubt as to what extent zinc contributes to the plaque inhibitory properties of sanguinarine-zinc mouthwashes. The interaction of zinc and sanguinarine has been investigated in some detail by one group and they concluded that the effect on plaque was more determined by sanguinarine concentration than by the presence or absence of zinc.[101] However, the addition of zinc did produce a slight enhancement of its effects.

In conclusion, sanguinarine appears to be an effective plaque inhibitory agent but is less effective in this regard than chlorhexidine. Also, unlike chlorhexidine, it is not able to prevent the development of gingivitis. Furthermore, the mouthwash is a much more effective plaque inhibitory agent than the toothpaste which may be devoid of activity. This may be due to the binding of other components in the toothpaste to the chemically reactive site of the sanguinarine molecule.

Propolis

Propolis is a naturally occurring bee product used by bees to seal openings in their hives.[115] It mainly consists of of wax and plant extracts and contains flavones, flavanones and flavanols. It has been used in homoeopathic remedies as an antiseptic, anti-inflammatory, antimycotic and bacteriostatic agent, and because of these properties it has been suggested as a constituent of a plaque-inhibitory mouthwash.

A double blind, parallel clinical study of the effectiveness of a propolis mouthwash has been carried out with negative and positive controls.[115] This showed that it had a very low level of clinical effectiveness and was not significantly better in inhibiting *de novo* plaque growth than the negative control. It does not therefore appear to have any use as a mouthwash.

Oxygenating agents

Oxygenating agents such as hydrogen peroxide, and buffered sodium peroxyborate and peroxycarbonate in mouthrinses have a beneficial effect on acute ulcerative gingivitis, probably by inhibiting anaerobic bacteria.[116] As obligate anaerobes are important in the development of gingivitis and periodontitis, these effects could be useful. The information relating to the value of these agents in suppressing supragingival plaque formation is limited although some retardation of plaque growth has been noted with the use of oxygenating mouthwashes.[117] In view of the importance of obligate anaerobic bacteria in the development of gingivitis and periodontitis these compounds deserve further investigation.[1]

The alcohol content of mouthwashes

Many mouthwashes contain significant quantities of alcohol and this may have a number of possible disadvantages. Firstly, it is important that they are not accidentally swallowed particularly by young children. In this regard, alcohol toxicity from this source has been reported.[118,119] Secondly, because of the known links between alcohol consumption plus tobacco smoking, and oral and pharyngeal cancer, it has been suggested that the frequent use of alcohol-containing mouthwashes might increase the incidence of this form of cancer. However, the evidence for this appears to be very weak, mainly because the statistical tests applied to test the strength of association are effected by the confounding effects of known aetiological factors such as tobacco smoking and alcohol consumption in the subjects studied.[120–123] Thirdly, it has been suggested that the use of alcohol-containing mouthwashes may increase the alcohol content of exhaled breath and could thus change the readings of the police breath test.[124] However, this effect was found to be transient. Finally, alcohol-containing mouthwashes have been shown to reduce the hardness of composite and hybrid-resin restorations and these effects seem to relate to the percentage alcohol content of the mouthwash.[125] It has also been found that composite resins soaked in alcohol-containing mouthwashes gain more weight that those soaked in alcohol-free mouthwashes.[126] This suggests that some component of mouthwash, probably alcohol, is absorbed into the resin and may be responsible for the softening effect. However, one study has found that either alcohol-containing or alcohol-free mouthwashes reduced the hardness of composite resin and glass ionomer cement.[127] In addition, it has been found that alcohol-containing mouthwashes may alter the colour of some hybrid composite resins.[128]

The possible uses of anti-plaque mouthwashes

The main uses of anti-plaque mouthwashes are as follows:

1. To replace mechanical toothbrushing when this is not possible in the following situations:
   - After oral or periodontal surgery and during the healing period (fig. 4)
   - After intermaxillary fixation used to treat jaw fractures or following cosmetic jaw surgery
   - With acute oral mucosal or gingival infections when pain and soreness prevents mechanical oral hygiene
   - For mentally or physically-handicapped patients who are unable to brush their teeth themselves. However, these patients may also not be able to use a mouthwash so that swabbing the gingival margins by a care worker may be the only option. This may not necessarily be easier for the care worker to carry out than brushing. The long-term use of effective agents has the major disadvantage of causing tooth staining.

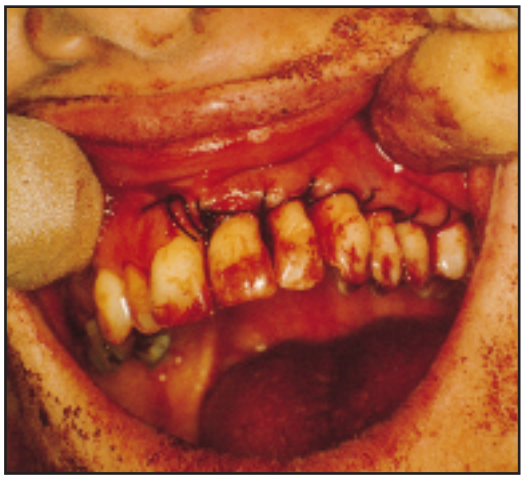

Fig. 4 A patient undergoing periodontal surgery seen just at the completion of suturing. This patient will be unable to brush this area for about 2 weeks and plaque control will need to be maintained by the use of an effective anti-plaque mouthwash such as chlorhexidine

2 As an adjunct to normal mechanical oral hygiene in situations where this may be compromised by discomfort or inadequacies:

- Following subgingival scaling and root planing when the gingivae may be sore for a few days. The use of a mouthwash is usually only necessary for about 3 days in this situation.
- Following scaling when there is cervical hypersensitivity due to exposed root surface. Its use needs to be combined with measures to treat the hypersensitivity since the duration for the use of the mouthwash should usually not exceed 2 weeks to avoid tooth staining. However patients vary considerably in the amount of staining they experience and some may have staining within a few days and others show little after 1 month's use.
- Following scaling in situations where the patient's oral hygiene remains inadequate. The inadequacy needs to be remedied quickly since the duration of the mouthwash use should not exceed 2 weeks in order to avoid staining. It would be better to have a suitable antibacterial agent which does not cause significant staining in a toothpaste or pre-brush rinse, such as triclosan, for this purpose in view of the above restriction.

Anti-plaque mouthwashes have no place in the treatment of existing periodontal disease, either gingivitis or periodontitis, since they cannot either reach the subgingival environment or penetrate thick layers of established plaque. In these situations they should only be used after supra- and subgingival scaling has been carried out, rendering the tooth surfaces clean, in order to maintain this situation for a short period when the soreness of the gingiva may prevent effective mechanical plaque control.

All patients using chlorhexidine mouthwashes for short periods should be told to avoid drinking tea, coffee and red wine over this period in order to minimise the tooth staining. Such mouthwashes should generally not be used for smokers since they would cause major tooth staining in this situation.

While many of the agents discussed above have significant plaque inhibitory activity when compared with an inactive placebo the extent of this varies among different agents and different formulations eg mouthwash and toothpaste. Many of the more effective agents share the side effect of producing tooth staining which limits their longer term use. Only one group of agents, the bisguanides of which chlorhexidine is the most effective, produce appreciable anti-plaque activity and thus are able to prevent the development of an experimental gingivitis. This is because they combine substantivity (oral retentiveness) with antibacterial activity and thus remain active in the mouth for long periods after their use. Effective anti-plaque agents must have these combined properties to work. The bisguanides are therefore the only group of mouthwashes with therapeutic efficiency and all the others are normally compared against this yardstick. Other agents have plaque inhibitory effects without substantivity and thus are not therapeutically effective. They can at best be used as adjuvants to mechanical cleaning measures such as toothbrushing.

Two other experimental agents, delmopinol and salifluor, also hold promise in this regard, and both of these have anti-inflammatory and hence anti-gingivitis effects in addition to plaque inhibitory effects.

Assessing manufacturers' claims about mouthwashes

The degree of effectiveness of a commercial mouthwash is very variable and depends on the composition of both the active and various additional agents within the mouthwash. Their characteristics are best assessed under the following headings:

- Range of antibacterial activity against the various plaque bacteria
- Substantivity to the oral surface
- Possible anti-inflammatory effect
- Acceptable taste
- Ability to promote fresh mouth sensation.

They can be grouped into three categories on the basis of these properties:

Group A. These are mouthwashes with good substantivity and antibacterial spectrum and thus have good anti-plaque effects. The only agents with these properties are the bisguanides, the best of which is chlorhexidine. These can be used to replace mechanical cleaning methods for short periods when this is not possible. The main drawback of the bisguanides is staining which is strongly linked to their substantivity. It precludes their prolonged use. Commercial chlorhexidine mouthwashes which do not produce staining are inactive usually because the active chlorhexidine molecules have been bound to another constituent of the mouthwash.

Two other agents, salifluor and delmopinol, either achieve or come close to achieving these properties but probably by rather different mechanisms to chlorhexidine.

Group B. These are agents with little or no substantivity but with a good antibacterial spectrum. Therefore, they have plaque inhibitory effects but lack true anti-plaque effects. They thus cannot be used to replace toothbrushing but can be used as adjuvants to mechanical cleaning. They include cetyl pyridinium chloride, the essential oil/phenolic mouthwash, Listerine, and triclosan. In the case of triclosan additional constituents such as zinc citrate or a co-polymer can enhance or prolong its plaque inhibitory effects possibly in the case of the latter by increasing its retention time in the mouth when used as a contistuent in mouthwashes or toothpastes.

Group C. These are antiseptic mouthwashes that have be shown to have antibacterial effects *in vitro* but in clinical studies have been shown to have either varying plaque inhibitory effects from moderate to low or no statistical difference from the negative control. These include hexetidine (Oraldene), povidone iodine, oxygenating agents and the natural product sanguinarine (Veadent) which is a benzophenanthridine alkaloid. These would have limited or no adjuvant effects when combined with mechanical cleaning and therefore cannot be recommended for this purpose.

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