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Biofilm reduction and staining potential of a 0.05% chlorhexidine rinse containing essential oils

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Hofer D, Meier A, Sener B, Guggenheim B, Attin T, Schmidlin PR. Biofilm reduction and staining potential of a 0.05% chlorhexidine rinse containing essential oils.

Abstract: *Objectives:* To assess the biofilm reduction and discolouration potential of a new 0.05% chlorhexidine (CHX) digluconate solution, containing additional essential oil and alcohol components, compared with that of standard control CHX solutions (0.05% and 0.2% CHX). *Methods:* The potential to reduce total viable counts of growing mixed microbial populations was examined using the Zurich biofilm model. Biofilms were created on sterile pellicle-coated hydroxyapatite discs and exposed to test substances at different time points. After 64.5 h, mean colony-forming units and SDs were determined. Colour change measurements using light reflection analysis were carried out on saliva preconditioned bovine dentin and enamel samples, as well as on composite and glass ceramic restorative materials, after successive immersions in a standardized tea brew and the CHX solutions. *Results:* The test solution was able to reduce biofilm formation by 3 log steps compared with a negative (water) control. This was significantly less effective than the standard control CHX solutions, which reduced viable counts by 6 log steps. Both the test and control solutions exhibited staining on all surfaces. Staining was most pronounced on dentin, followed by enamel and to a significantly lesser degree on the restorative materials. Furthermore, the staining caused by the test solution on these restorative materials was generally lower than that caused by the control solutions. *Conclusions:* The test solution exhibited an antimicrobial activity. The composition, however, seems to hamper its effectiveness. Accordingly, it produced statistically significant, although by trend less, staining on restorative materials.

Key words: antimicrobial effectiveness; biofilm; Chlorhexidine; discolouration; *in vitro*

Introduction

Various species of bacteria found in mature dental biofilms are recognized to be the contributing factors to both periodontal diseases and caries development (1, 2). It has been shown that meticulous daily plaque control will prevent disease initiation, stop progression of the disease process and, combined with professional debridement, allow surrounding tissues to return to a healthy state (3–5). Unfortunately, inadequate daily removal of bacterial plaque and biofilm is widespread (6–8). Even well-trained patients may miss hard-to-reach areas around posterior teeth or marginal gingiva. Additionally, people with malpositioned teeth, bridgework or orthodontic appliances and especially elderly people with physical or mental limitations may find brushing and interdental cleaning extremely difficult (9). Antimicrobial rinses are, therefore, often recommended as an adjunctive homecare procedure. Of all antimicrobials studied and currently used, chlorhexidine (CHX) has long been recognized as the most effective for inhibiting plaque, preventing gingivitis and displaying a well-documented anti-caries effect (10–13). In repeated studies, depending upon the concentration used, CHX has been shown to prevent plaque accumulation, with two marked negative side effects: surface staining and altered taste perception (14). Both side effects are reversible upon discontinuation of use, but remain a major stumbling block in regard to patient compliance.

Researchers, and industry, have put a lot of effort in developing formulations that reduce the negative side effects while maintaining the powerful antimicrobial effect of CHX. However, because of the strong positive charge, CHX loses its antimicrobial effect rapidly when combined with organic or inorganic molecules (15, 16). Only lower concentrations appear to cause less stain, or less rapid staining, but at the cost of efficacy (17–19).

This *in vitro* study was designed and executed in two parts, first to determine the antimicrobial efficacy of a new 0.05% CHX digluconate solution containing essential oil and alcohol components (Parodentosan®; Tetan AG, Ramlinsburg, Switzerland) and then to assess its staining potential compared with that of standard control CHX solutions. The null hypothesis tested was that the test product I) is as effective in reducing biofilm formation as the control solutions and II) that it will cause staining identical to that caused by control solutions on enamel, dentin and selected restorative materials.

Study population and methodology

Antimicrobial solutions

One test and four control solutions were examined in Experiment 1 for their ability to inhibit biofilm formation (Table 1). One test and three control solutions were used in the staining experiment (Table 2). The test solution is readily available over the counter in Switzerland. Separate control solutions were mixed for Experiments 1 & 2. In Experiment 1, non-additive control CHX dilutions were mixed from the same batch and distilled water was used as a negative control. Similarly, all the control CHX dilutions used in Experiment 2 stemmed from one batch, with de-ionized water used as a negative control.

Table 2. **Experiment 2 solutions and materials**

Type	Brand name	Manufacturer
CHX 0.05% test solution with sage, menthol, myrrh 15 Vol.% ethanol	Parodentosan	Tentan AG, Ramlinsburg, Switzerland
CHX 0.05% positive control solution		Kantonsapotheke Zurich, Switzerland
CHX 0.2% positive control solution		Kantonsapotheke Zurich, Switzerland
De-ionized H_2O negative control solution		Produced in-house
Micro-filler	Tetric A2	Ivoclar, Schaan, Liechtenstein
Nano-filler	Filtek Supreme XT A2B	3M ESPE, Seefeld, Germany
Glass ceramic	Empress CAD A2	Ivoclar, Schaan, Liechtenstein

Table 1. **Experiment 1 solutions**

Type	Brand name	Manufacturer
CHX 0.05% test solution with sage, menthol, myrrh 15 Vol.% ethanol	Parodentosan®	Tentan AG Ramlinsburg, Switzerland
CHX 0.05% positive control solution 15 Vol.% ethanol		Sigma Chemical Co St. Louis, MO, USA
CHX 0.2% positive control solution 15 Vol.% ethanol		Sigma Chemical Co St. Louis, MO, USA
CHX 0.2% positive control solution		Sigma Chemical Co St. Louis, MO, USA
Distilled H_2O negative control solution		Produced in-house

Experiment 1: Biofilm formation

A detailed account of the materials and methods of biofilm formation has been previously presented elsewhere (20); therefore, only a synopsis is provided in this article.

Biofilms

Biofilms contained *Actinomyces naeslundii* OMZ 745, *Veillonella dispar* OMZ 493, *Fusobacterium nucleatum* OMZ 596, *Streptococcus sobrinus* OMZ 176, and *Streptococcus oralis* OMZ 607 and *C. albicans* OMZ 110. A total of 36 pellicle-coated hydroxyapatite discs (Ø 9 mm, Clarkson Chromatography Products, Inc, South Williamsport, PA, USA) in 24-well polystyrene cell culture plates were covered with 1.6 ml of processed whole unstimulated saliva + modified fluid universal medium (mFUM), supplemented with 67 mmol l^{-1} Sørensen's buffer (38% v/v, final pH 7.2) containing carbohydrate (21). The carbohydrate concentration in stock solutions of mFUM was 0.3% (w/v) and consisted of either glucose (biofilm cultivation from 0 to 16.5 h) or a 1:1 (w/w) mixture of glucose and sucrose (biofilm cultivation from 16.5 to 64.5 h). Wells were inoculated with mixed cell suspensions (200 µl) prepared from equal volumes of each species adjusted to an OD 550 and incubated anaerobically at 37°C. Medium was changed after dipping (see below) at 16.5 and 40.5 h by aspirating spent medium and adding back fresh medium.

Evaluation of antimicrobial activities of test solutions

Biofilm-covered discs were immersed for 1 min in 1 ml of test solution, and then rinsed gently by dipping in physiological saline (3 × 2 ml). The biofilms were exposed to the test and control substances (n = 9) at 16.5, 20.5, 24.5, 40.5, 44.5 and 48.5 h. After the last treatment, the biofilms were incubated undisturbed and harvested at 64.5 h by vigorous vortexing in physiological saline (1 ml).

Aliquots of harvested biofilm were sonified, diluted and spiral plated onto Columbia agar base (Oxoid, Ltd., Basingstoke, Hamps., UK) containing 5% (v/v) haemolysed human blood (CBA) and incubated anaerobically at 37°C. Colony-forming units (CFUs) were counted 72 h after plating with the aid of a stereomicroscope.

Statistics

Statistical analyses of the effects of different treatments on total biofilm populations were performed using log10-transformed *total* CBA CFUs. Skew distributions of the values measured for most products and different variances of the solutions examined required non-parametrical statistical tests. Overall, statistical analyses within defined groups of products were performed using the Kruskal–Wallis procedure as implemented in the program StatView II (Abacus Concepts, Inc., Berkeley, CA, USA). As a result of the multiple test situations, Bonferroni's correction was applied.

Experiment 2: Staining potential

Stain formation

A standardized *in vitro* method for reproducing stain in the presence of CHX was followed (19). A standard tea solution (Marks and Spencer extra strong, London, UK) was prepared by boiling 8 g of tealeaves in 800 ml of distilled water for 2 min. The solution was allowed to cool in a refrigerator at 4°C for 30 min and the infusion filtered through gauze to remove the tealeaves. Finally, the tea solution was kept at 37°C during the experiment.

This investigation used different tooth and restorative materials (Table 2) as test specimens, in place of clear acrylic blocks. These specimens were prepared as follows: the crowns from sixty-four caries-free bovine mandibular incisors of 2.5-year-old animals were mechanically separated. The labial aspects were sectioned (enamel n = 32) and the middle dentine (n = 32) prepared using a PD-Max grinder (Streuers GmbH, Birmensdorf, Switzerland) at 300 revolutions per minute, under water cooling with SiC paper 500 grit (Merck, Dietikon, Switzerland) followed by p1000 grit (DIN 69176; grit size 18 µm) wet. The specimens were then hand-polished to a standardized reproducible flat surface [ISO/TR 1994]. The restorative materials were prepared by placing each material (n = 32/material) in 13-mm-round, 3-mm-thick Teflon forms. In a first curing phase, the forms were only half filled and cured with a UV light source (blue phase, Ivoclar, Schaan, Liechtenstein) on 4 points within the circle radius for a total of 40 s. A second curing phase was performed after the forms were fully filled, on 6 points, for a total of 60 s. The specimens were then placed in a broad beam light-curing chamber (Spectramat, Ivoclar, Schaan, Liechtenstein) and cured for a third time, for 5 min.

All specimens were embedded in optically clear epoxy resin (Stycast®; Emerson & Cuming, Waterlo, Belgium), which was mixed in the proportion Stycast 1266 Part A 15 g and Part B 4.2 g for 90 s by hand, then for 19 min under a vacuum pump to generate a bubble-free mass. The outer dimensions of the samples measured 20 mm in diameter, to fit the optical lens of a

Konica/Minolta spectrophotometer [CM-508d, Konica Minolta Photo Imaging (Schweiz) AG, Switzerland].

The five specimen materials were divided into four groups of eight specimens each. They were covered and bathed in a pooled stimulated human saliva (gathered from volunteers at 7:45 and 11:45 a.m. on the day of testing, without ingestion of food for at least 2 h before sample collection and held between cycles at 37°C) for 2 min at 37°C, then rinsed four times consecutively with 2 ml of de-ionized water. Each of the eight specimens were then covered and bathed by groups in one of the three CHX solutions applied in this experiment (Table 1), or with a de-ionized water negative control at 37°C for another 2 min, before being rinsed again four times consecutively with 2 ml of de-ionized water. The specimens were then covered and bathed in the standard tea solution and re-incubated for 1 h at 37°C. A final rinse of four consecutive 2 ml washes was performed and the specimens were dried with compressed air and measured for luminosity using the CIELAB (L*a*b*) colour system on a daylight, D65/10°, scale. The saliva/CHX/tea bath cycle was repeated six times over 11 h.

Stain determination.

A baseline L*a*b* reading had been taken prior to the start of the saliva/CHX/tea baths and the changes in L* (luminosity), a* (red-green axis) and b* (yellow-blue axis) were recorded using a Konica/Minolta spectrophotometer (CM-508d, wavelength range 400–700 nm) and fed directly into a computer (MacIntosh G4, Apple, Cupertino, CA, USA).

The overall colour difference was calculated as:

$$\Delta E^*_{ab} = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

where,

$$\Delta L^* = L^*_{interval} - L^*_{baseline};$$
$$\Delta a^* = a^*_{interval} - a^*_{baseline};$$
$$\Delta b^* = b^*_{interval} - b^*_{baseline}$$

Statistics

Two-way ANOVA was used to compare differences in colour of the various substrate materials occurring under the influence of the three different CHX solutions and de-ionized water control. Mean values were compared with Scheffe's multiple comparison test at the 0.05 level of significance (StatView, Abacus Concepts, Inc.).

Results

Experiment 1

The results of the biofilm experiment are summarized in Table 3. The distilled water control showed the highest number of viable microorganisms ($1.5 \times 10^8 \pm 5.3 \times 10^7$). The test solution was able to reduce the biofilm growth by 3 log steps ($6.4 \times 10^5 \pm 1.1 \times 10^6$; $P < 0.05$). The CHX control solutions showed almost complete inhibition of bacterial growth, i.e. a reduction of 6 and 7 log steps respectively.

Experiment 2

The results are summarized in Table 4. All substrate surfaces tended to become darker and more discoloured over time. This darkening (ΔL*) and discolouration (ΔE*) were significantly more pronounced on the enamel and dentin samples, compared with that on the three restorative materials. Whereas contact with the test and positive control solutions did not produce significantly more staining than contact with water before the tea bath on either the enamel or dentin specimens, the change in ΔL* caused by the 0.05% control CHX solution was significantly higher than that produced by either the 0.2% control CHX solution or the test solution. For the dentin samples, the colour change along the red-green axis (Δa*) was significantly greater for both control CHX solutions than the change displayed by the test solution. However, there were no significant changes in ΔL* or ΔE* for these dentin substrate samples, when compared with those caused by the water control.

On the micro-filler and ceramic substrates, the ΔL* was significantly greater after contact with the pure CHX solutions, compared with that after contact with water or the test solution. However, all the three CHX solutions produced a level of staining significantly higher than that of the water control on the nano-filler, with the test solution showing a tendency,

Table 3. **Mean colony-forming units after exposure to the test and control substances ± 1SD**

Distilled water 15% ethanol	0.05% CHX, sage, menthol, myrrh 15% ethanol	0.05% CHX 15% ethanol	0.2% CHX 15% ethanol	0.2% CHX
$1.5 \times 10^8 \pm 1.1 \times 10^{6ABCD}$	$6.4 \times 10^5 \pm 1.1 \times 10^{6AEFG}$	$3.9 \times 10^2 \pm 5.2 \times 10^{2BE}$	$5.6 \times 10^1 \pm 1.1 \times 10^{2CF}$	$2.1 \times 10^1 \pm 0^{DG}$

Identical upper case alphabets indicate a significant difference ($P < 0.05$).

Table 4. **Mean changes in luminosity (ΔL*), red-green axis (Δa*), yellow-blue axis (Δb*) and overall colour (ΔE*) ± 1SD**

		De-ionized Water	CHX 0.05%	CHX 0.2%	0.05% CHX sage, menthol, myrrh, 15% ethanol
Enamel	ΔL*	−8.5 ± 3.5	-10.1 ± 0.7^{AB}	-8.9 ± 2.7^{A}	-8.6 ± 2.2^{B}
	Δa*	6.1 ± 1.9	6.8 ± 0.8	7.5 ± 1.1	6.7 ± 1.0
	Δb*	12.6 ± 3.6	14.4 ± 1.2	15.4 ± 1.8	13.2 ± 1.1
	ΔE*	16.5 ± 4.8	18.8 ± 1.1	19.5 ± 2.3	17.3 ± 2.2
Dentin	ΔL*	−11.3 ± 4.0	−15.0 ± 2.9	−14.0 ± 2.8	−10.9 ± 3.1
	Δa*	7.1 ± 1.5^{AB}	9.8 ± 1.5^{A}	9.6 ± 1.4^{B}	8.5 ± 1.9
	Δb*	15.0 ± 3.0	16.9 ± 3.3	17.0 ± 2.7	13.9 ± 4.5
	ΔE*	20.5 ± 3.1	24.7 ± 4.0	24.2 ± 3.1	19.8 ± 5.1
Composite (micro-filler)	ΔL*	-3.0 ± 1.7^{AB}	-5.8 ± 1.0^{A}	-5.6 ± 0.5^{B}	−4.0 ± 2.2
	Δa*	1.7 ± 0.9^{AB}	3.7 ± 0.4^{A}	3.6 ± 0.4^{B}	2.5 ± 1.6
	Δb*	3.9 ± 1.1^{AB}	7.9 ± 0.8^{A}	8.3 ± 0.6^{B}	5.8 ± 1.6^{B}
	ΔE*	5.2 ± 2.0^{AB}	10.5 ± 1.1^{AC}	10.6 ± 0.7^{BD}	7.5 ± 2.0^{CD}
Composite (nano-filler)	ΔL*	-3.0 ± 0.9^{ABC}	-5.6 ± 0.7^{A}	-5.3 ± 0.8^{B}	-4.3 ± 1.6^{C}
	Δa*	1.8 ± 0.5^{ABC}	3.6 ± 0.6^{A}	3.6 ± 0.6^{B}	2.9 ± 1.1^{C}
	Δb*	3.4 ± 0.8^{AB}	7.4 ± 0.9^{A}	7.4 ± 0.8^{B}	5.7 ± 1.1^{B}
	ΔE*	4.9 ± 1.2^{ABC}	9.9 ± 1.2^{A}	9.8 ± 1.1^{B}	7.9 ± 2.1^{C}
Glass ceramic	ΔL*	-2.8 ± 2.0^{AB}	-5.5 ± 0.8^{A}	-4.9 ± 0.7^{B}	−4.6 ± 1.6
	Δa*	1.9 ± 1.0^{ABC}	3.7 ± 0.8^{A}	3.5 ± 0.5^{B}	3.5 ± 0.6^{C}
	Δb*	3.1 ± 1.2^{ABC}	6.7 ± 1.2^{A}	6.3 ± 1.4^{B}	6.0 ± 0.5^{C}
	ΔE*	4.9 ± 1.2^{ABC}	9.4 ± 1.5^{A}	8.8 ± 1.5^{B}	8.4 ± 0.8^{C}

Identical upper case alphabets (to be read horizontally) indicate a significant difference ($P < 0.05$).

although not statistically significant, for lesser staining also on this substrate.

On the nano-filler and ceramic specimens, significant shifts in ΔE* occurred after exposure to both the test and control CHX solutions. Moreover, the test solution displayed a tendency, although not statistically significant, for less staining. However, on the micro-filler substrate, staining was significant for the specimens exposed to the control CHX solutions, whereas the test solution only displayed a statistically insignificant difference to that recorded by the specimens exposed to the water control.

Discussion

This study assessed the capability of a new 0.05% CHX digluconate solution containing menthol, myrrh, sage and alcohol components to inhibit biofilm formation, as well as to determine its staining potential compared with that of standard CHX solutions.

These standard control solutions varied between the two experiments and reflect the separate aims of each experiment. Experiment 1 tested for biofilm suppression. The test solution was benchmarked against dilutions of a non-additive control solution, with and without equimolar alcohol content, mixed in-house to ensure purity. Experiment 2 tested for staining potential and benchmarked the test solution against dilutions of a private-label control solution that is commonly used, when indicated, by patients at the dental school and known to cause severe staining. Ethanol is neither a standard ingredient in this CHX rinse nor was it mixed in for this experiment; because although its inclusion may have an effect on efficacy, its absence has neither been shown nor suspected of having an influence on staining. Hence, only two positive control solutions were used in Experiment 2.

The results showed that the test solution had a significant antibacterial effect on the experimental biofilm; however, a better efficiency with pure CHX solutions with or without equimolar alcohol content was observed. The latter solution almost completely prevented biofilm formation and reduced the biofilm growth by 6–7 log steps. Therefore, the first null hypothesis was rejected.

In an earlier study of similar design (22), a non-CHXmouth rinse containing menthol, thymol, methyl salicylate, eucalyptol, and benzoic acid (Listerine®, Johnson & Johnson Healthcare Products, Skillman, NJ, USA) displayed biofilm inhibition properties similar to those observed with the CHX test solution in the current study. Both solutions reduce biofilm formation by 3 log steps. In Listerine, ethanol is present in concentrations of 21.6% in the flavoured products and 26.9% in the original antiseptic formulation. The test solution contains 15-volume percent ethanol.

For the biofilm formation, hydroxyapatite discs were chosen to simulate the enamel structure. A previous study assessed

biofilm growth on other substrates, i.e. human enamel and different composite resin materials (23). That study showed that surface roughness influenced initial biofilm adherence during the initial adherence phase (20 min), but differences vanished following growth and maturation (16.5 h).

The staining experiment showed that the test and control rinses exhibited staining on all surfaces. Staining was most pronounced on dentin, followed by enamel and to a significantly lesser degree on restorative materials. The staining caused by the test solution was generally lower than that caused by the control CHX solutions on the restorative materials. In terms of the materials selected in this experiment, substrates relevant for the oral environment under clinical conditions, e.g. enamel, dentin, composite resin and ceramic material, were chosen.

Bovine enamel and dentin samples are commonly used as proxies for their human counterparts, as they are readily attainable in sufficient quantities for a study of this nature. The three restorative materials chosen for inclusion in this study were representative of their class: a micro-filler hybrid, a nano-filler hybrid and a glass ceramic. All surfaces were polished with the same grit (P1000) to obtain comparable surfaces. As Stober *et al.* (24) pointed out, this does not necessarily represent a clinical level of polishing, which might have reduced the level of staining produced. However, it does provide a standard, and observable, level to which the finding of this study can be compared with the findings of other studies.

After polymerization shrinkage (in the case of micro-filler and nano-filler hybrids) and secondary caries, plaque accumulation and colour stability are most often mentioned as major problems in tooth-coloured restorations. As CHX is often prescribed for patients with both an elevated caries risk and persistent periodontal problems, the possibility or probability of staining takes on a certain level of importance.

Stober *et al.* (24) also offer a review of six studies evaluating the level of overall colour change (ΔE*) that can be detected by the human eye. Their conclusion, which is consistent with Fay *et al.* (1999), Abu-Bakr *et al.* (2000) and Lee & Powers (2004), is that a value of greater than 3.3 is visible to the human eye and must be considered unacceptable (25–27).

Based on these parameters, the results of this study show that contact with saliva and tea alone was enough to cause clinically unacceptable colour changes on enamel, dentin, micro-filler, nano-filler and ceramic restorative materials. This finding was unexpected and is in contrast to findings of other studies that found significantly less staining when specimens are subjected to saliva, water and tea than when CHX is added to the submersion protocol (28, 29). However, Carpenter *et al.* (30) also found greater staining on their specimens (hydroxyapatite discs pretreated with parotid saliva to form an acquired pellicle) when exposed to tea alone than when exposed to CHX and tea together. Thus, there is no consensus concerning this issue. Methodological aspects in terms of substrate to be stained, pellicle formation and teas used and immersion protocols may cause differences in the outcomes.

The second null hypothesis that the test solution will stain, as measured by overall colour change, as heavily as an equivalently dosed control CHX solution was confirmed on a statistical level on all test substrates except the micro-filler. There was, however, a displayed tendency to lesser overall colour change on the other two restorative materials as well as the enamel and dentin specimens. Both the micro-filler and ceramic restorative materials also displayed statistically less darkening.

This tendency towards lesser staining/darkening by essential oil compounds has also been observed in at least two comparative trials between CHX and Listerine (31, 32). The additives in the test solution; menthol, myrrh and sage, are similar to those found in Listerine, where they have been proven to be clinically effective anti-gingivitis and, although to a lesser degree, anti-plaque agents that do not promote extrinsic tooth stain (32, 33). So whereas their inclusion in the test solution inactivated the CHX to a significant degree, as shown in the biofilm experiment, the mouth rinse still displays a statistical significance when compare with the water control in reducing biofilm growth.

In general, it must be remembered that the staining protocol, as explained in the Methods section, was designed to provide maximum staining potential in an *in vitro* setting. As such, it has a restricted applicability to a clinical situation where different types of tea (in different concentrations, temperatures, possibly the presence of milk) as well as other dietary chromogens and the influence of tooth brushing and tooth pastes all play a role in the accumulation of extrinsic stains (19, 26). However, such protocols do allow comparisons between test solutions and test substrates, and point out staining tendencies that might warrant further investigation.

An unexpected observation was that the 0.05% control CHX solution produced both overall colour change and darkening to the same order of magnitude as the 0.2% solution. CHX has been shown over the years and in many studies to be dose dependent (34–36). However, a careful analysis of previous dose and staining trials revealed one study that provided a similar result, whereby a 0.1% CHX solution stained significantly more than a 0.2% solution (19). The authors of this study did not address this result in their discussion of their findings. In this study, too, the factors behind this result are not clear. Possible explanations include mechanisms of competitive binding

to the pellicle, saturation of receptors or changes in valency of the dicationic molecule. Further research is needed to clarify this issue.

Conclusions

The test solution under investigation showed a significant reduction of biofilm formation. However, this action was less pronounced than that in the pure CHX controls with or without alcohol. The test solution displayed significantly less staining on a micro-filler composite restorative material than either a 0.05% or 0.2% control CHX solution. It caused, however, an overall colour change on enamel, dentin, nano-filler composite and ceramic restorative materials. Although not statistically significant, it was slightly less pronounced than the staining caused by the control CHX solutions tested. In general, the restorative materials displayed significantly less colour change and loss of luminosity after having been bathed in saliva, CHX and tea than did the enamel and dentin substrates tested under the same conditions.

Recommendations for future research

1 Clinical studies should be carried out to determine both the *in vivo* anti-plaque and anti-gingivitis efficacy of this CHX formulation, as well as its actual staining level when outside factors such as diet and daily oral hygiene are factored in.

2 *In vitro* studies should be performed to determine which additive(s) found in the test solution impede its efficacy compared with a 'pure' CHX solution, as well as to determine if the test solution reduces plaque formation because of its CHX component or essential oil components or any combination thereof.

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