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Short-term anti-plaque effect of two chlorhexidine varnishes

Jan Cosyn[1], Iris Wyn[1], Tim De Rouck[2], Kris Collys[2], Peter Bottenberg[2], Stefan Matthijs[1] and Mehran Moradi Sabzevar[1]

Departments of [1]Periodontology and [2]Restorative Dentistry, School of Dental Medicine, Free University of Brussels (VUB), Brussels, Belgium

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Abstract

Background: Chlorhexidine (CHX) varnishes have been mainly used for the prevention of caries in high-risk populations. Reports regarding their anti-plaque effect on a clinical level are limited to non-existing as opposed to their microbiological impact on plaque formation.

Aim: The aim of this preliminary investigation was to evaluate the anti-plaque effect of two CHX varnishes applied on sound enamel in relation to a positive control, a negative control and to one another.

Methods: Sixteen healthy subjects volunteered for this randomized-controlled, single-blind, four-treatment–four-period crossover-designed clinical trial. A 3-day plaque re-growth model was used to determine de novo plaque accumulation following CHX rinsing, Cervitec® application, EC40® application and no therapy. The amount of plaque was measured using the Quigley and Hein plaque index and ''automatic image analysis'' (AIA).

Results and Conclusions: Varnish treatment resulted in significantly higher plaque levels than CHX rinsing irrespective of the varnish that was used ($p \leqslant 0.002$), implying that the latter is likely to remain the gold standard as an anti-plaque agent. However, highly significant differences were also found in favour of both varnish systems when compared with no therapy ($p < 0.001$), which indicates that varnish treatment is an effective means of inhibiting plaque formation in a short time span. Cervitec® exhibited slightly, yet significantly, higher plaque levels in comparison with EC40® as determined by AIA ($p = 0.006$). Large-scale trials with a longer observation period are necessary to substantiate these results.

Key words: anti-plaque effect; automatic image analysis; chlorhexidine varnish; clinical study

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Chlorhexidine (CHX) is still considered as the gold standard in chemically controlling plaque accumulation. A number of vehicula delivering this antiseptic have been developed. The consideration that professionally applied dental varnishes overcome the non-compliance of the patient as opposed to mouth rinses makes them an appealing vehicle for CHX delivery. Today, essentially two CHX varnishes are available for clinical purposes: the volatile Cervitec® varnish (Vivadent, Schaan, Liechtenstein), containing 1% CHX and 1% thymol as antimicrobial agents, and the EC40® varnish (Certichem, Nijmegen, the Netherlands), containing 35% CHX. So far, these varnishes have been mainly used for the prevention of caries in high-risk populations. Indeed, repeated CHX varnish applications can suppress *Streptococcus mutans* in dental plaque, thereby lowering its cariogenic potential (Schaeken et al. 1991, 1994, Ie & Schaeken 1993, Twetman et al. 1995, Twetman & Petersson 1997, Madlena et al. 2000, Araujo et al. 2002, Gerardu et al. 2003). In this regard, Attin et al. (2003) compared both varnish systems with one another and concluded that EC40® generates a higher decrease of *S. mutans* than Cervitec® in plaque and saliva. Even though these microbiological effects have been extensively documented, the impact of repeated varnish applications on caries incidence remains confusing: indeed, some studies confirmed (Bratthall et al. 1995, Fennis-Ie et al. 1998, Joharji & Adenubi 2001, Baca et al. 2002, 2004), whereas others refuted a decrease of caries incidence (Jenatschke et al. 2001, de Soet et al. 2002).

In vitro studies by Petersson et al. (1992) designated putative periodontopathogens as being the most sensitive bacteria to the CHX–thymol varnish, thereby creating a promising role for CHX varnishes in the prevention and treatment of periodontal diseases. Yet, an intensive application mode seems mandatory in order to achieve a long-term beneficial effect on gingival health. Indeed, the effects on gingival parameters have not been very convincing following a single varnish administration or even multiple applications within

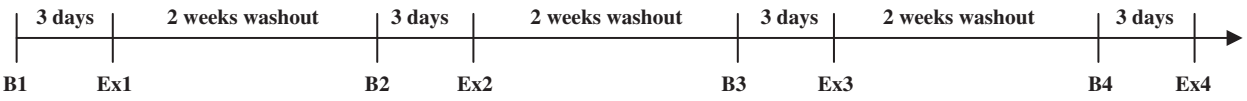

Fig. 1. Time sequence of the experimental design. B, baseline; Ex, examination point.

a short time span (Weiger et al. 1994, Øgaard et al. 1997). Only Valente et al. (1996) described significant favourable gingival effects following a thorough 3-month CHX varnish treatment regimen.

As an adjunct to scaling and root planing, the CHX–thymol varnish had little effect on subjects with good oral hygiene (Dudic et al. 1999). However, a significant short-term decrease of inflammatory mediators in the crevicular fluid could be recorded in orthodontic patients treated with the CHX–thymol varnish (Skold et al. 1998, Yucel-Lindberg et al. 1999).

The microbiological impact on dental plaque by both varnishes has been studied extensively (for a review, see Matthijs & Adriaens 2002). However, reports regarding their anti-plaque effect on a clinical level are very limited or non-existing. Hence, the objective of the present clinical study was to evaluate the anti-plaque effect in a short time span of these CHX varnishes applied on sound enamel in relation to a positive control, a negative control and to one another.

Materials and Methods

Experimental design

Sixteen patients (10 males and six females; mean age of 32 years) volunteered for this randomized-controlled, single-blind, four-treatment–four-period crossover-designed clinical trial. They consulted, or were referred to, the Department of Periodontology of the Free University of Brussels (VUB) or a private periodontal practice.

All subjects were in good general health. Subjects having taken any medication 3 months prior to or during the trial were excluded. For obvious reasons of plaque accumulation, subjects wearing removable partial dentures or undergoing orthodontic therapy were excluded. Caries lesions and plaque-related periodontal diseases had to be fully treated prior to enrolment. All subjects signed informed consent and received a thorough oral prophylaxis by means of ultrasonic and manual scaling and polishing of all teeth. Overhanging margins of restorations were also removed prior to commencement of the trial.

At baseline, all participants received a code number corresponding to a given treatment sequence. A randomization scheme ensured that every subject received another sequence. Thereupon, plaque was disclosed using red Rondell Disclosing Pellets (Svenska®, Svenska Dental Instrument AB, Upplands, Väsby, Sweden) on all buccal surfaces of front teeth and pre-molars in both jaws, serving as the experimental areas. Crowned teeth, teeth presenting class V restorations and teeth with gingival recessions were not considered. A minimum of seven experimental teeth per jaw was required for enrolment. One pellet was used per jaw, and patients were allowed to rinse briefly for 10 s with 2.5 ml of tap water. Subsequently, all disclosed surfaces were thoroughly polished with a Nupro® Fine Polishing Paste (Ash, Division of Dentsply International Inc., York, UK) applied by a rotating rubber cup. If necessary, ultrafloss (Oral-B®, Oral-B Laboratories, London, UK) and/or extra-fine polishing strips (Hawe Neos®, Hawe Neos Dental, Bioggio, Switzerland) were used to meticulously remove all plaque remnants inter-proximally. Polishing was continued until a zero-plaque score was clinically reached as determined by the clinicians' judgement. The quality of plaque removal was not additionally reviewed by re-applying the disclosing agent. Front teeth and premolars were further air-dried and meticulously isolated from water and saliva prior to varnish administration.

Figure 1 presents the time sequence of the experimental design. Irrespective of the treatment modality, plaque was allowed to grow undisturbed for 3 days. Oral hygiene was ceased during this period of time, and subjects were asked to pay attention to their eating habits and to avoid chewing gum at all times. This plaque re-growth model was limited to 3 days mainly for ethical reasons, to avoid the risk of developing plaque-related gingivitis, and yet concurrently ensuring relevant preliminary data. At day 3, the amount of plaque was measured by using a plaque index (PI) and by making colour slides in order to perform ''automatic image analysis'' (AIA). A washout period of 2 weeks was given between two treatment modalities to avoid carry-over effects (Newcombe et al. 1995). To ensure blindness, one investigator was charged with the allocation to a treatment sequence, prophylaxis, polishing and varnish application, whereas another investigator, unaware of the treatment carried out, performed scoring, took colour slides and conducted AIA. The Ethical Committee of the University hospital in Brussels approved the study protocol.

Treatment

All participants were subjected in a randomized order to the following four treatment modalities: Cervitec® application on the buccal surfaces of front teeth and pre-molars, EC40® application on the same surfaces, CHX 0.2% rinsing (Corsodyl® mouthwash twice daily during 1 min.) serving as the positive control and no therapy serving as the negative control. Both varnishes were applied according to the prescriptions of their manufacturers: Cervitec® was left on the teeth, whereas EC40® was gently removed after 7 min. using a standard periodontal curet.

Examination criteria

At day 3, the amount of accumulated plaque was determined by two methods:

The first was by means of the Quigley & Hein (1962) PI scored chair side at six sites per tooth (mesial, central, distal; buccally as well as orally). The scores ranged from 0 to 5 based upon the following coronal plaque extension levels: 0 = no plaque; 1 = separate flecks of stained plaque at the cervical margin; 2 = thin continuous band of stained plaque up to 1 mm at the cervical margin; 3 = band of stained plaque between 1 mm and one-third of the crown; 4 = stained plaque at least one-third but less than two-thirds of the crown; and 5 = stained plaque covering at least two-thirds of the crown.

The second method involved taking colour slides to perform AIA (Moradi

Sabzevar 1996). Per tooth surface, a proportional score was given (percentage of the total surface covered by plaque). All slides were taken with a Nikon® Medical Nikkor 120 mm lens (Nikon Corporation, Tokyo, Japan) fitted on a Nikon® F-801 body (Nikon Corporation) with a standard mounted circular flashlight. Agfa® ISO 100 CT Precisa professional slide film (Agfa-Gevaert AG, Leverkusen, Germany) was used for all slides. Per tooth surface, one slide was made using hand-held photography. Special attention was paid to take the slide of the tooth as orthogonally as possible in the vertical as well as in the horizontal plane in order to minimize error. All slides were developed according to the manufacturers' instructions. Thereupon, the slides were digitized with a video camera (AxioCam MRc, Carl Zeiss, AG, Oberkochen, Germany) and the frame grabber of the KS100 (Zeiss) software. Image analysis was carried out semi–automatically with the KS400 (Zeiss) software, using a special macro described briefly as follows. First, the digitized colour image was converted into a grey image and the contrast was enhanced. Because of poor grey value contrast between gingiva and plaque, the tooth contour had to be drawn manually to enable discrimination (''cutlink'', grey value 255). The resulting binary image and the grey image were combined with a boolean operation (''binand'') and contrast was enhanced once more. This made it possible to discriminate plaque from plaque-free areas by performing grey value segmentation (''dislev''). The resulting binary image was compared with the original colour image and, if necessary, corrected either manually or by a scrap function (''binscrap'').

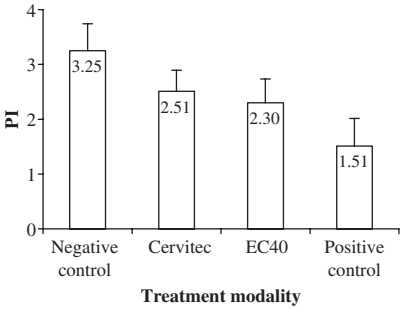

Fig. 2. Bar chart showing mean plaque scores per treatment modality as determined by the Quigley and Hein plaque index.

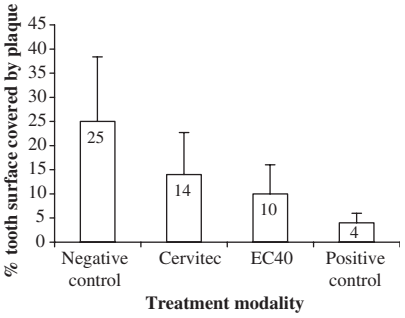

Fig. 3. Bar chart showing the mean proportion of the tooth surface covered by plaque per treatment modality as determined by automatic image analysis.

Calibration session

To ensure reliability of test results, the investigator charged with clinical assessments and AIA had to be calibrated for intra-examiner repeatability prior to commencement of the trial. With regard to calibration of the PI, three volunteers refraining from tooth brushing for 72 h were enrolled. Duplicate PI measurements ($n = 180$) were collected with an interval of 15 min. between the first and the second recording. Intra-examiner repeatability of AIA was based on duplicate analysis of 80 slides with an interval of 4 h in between. Both techniques were found to be highly reproducible (PI: Spearman's correlation: $r = 0.87$, $p < 0.001$; AIA: Pearson's correlation: $r = 0.99$, $p < 0.001$).

Statistical analysis

Data analysis was performed with the subject as the unit of analysis. Mean values for PI and AIA were calculated per treatment modality and per subject, serving as primary outcome variables. Mainly because of the limited sample size, a conservative statistical approach was chosen using a Friedman test to calculate an overall p-value based on mean subject PI and AIA data. If this value reached the 5% level of significance, Wilcoxon's signed ranks tests were applied to seek differences between treatment modalities by comparing them two by two. All resulting p-values were Bonferroni corrected by lowering the level of significance to 0.05/6 or 0.0083.

Results

Figures 2 and 3 show mean plaque levels sorted per treatment modality as determined by PI and AIA. The positive control encompassed the lowest mean plaque levels (PI: 1.51 ± 0.50; AIA: $4 \pm 2\%$), whereas the negative control was found at the other extreme (PI: 3.25 ± 0.49; AIA: $25 \pm 13\%$). Mean plaque levels of Cervitec® (PI: 2.51 ± 0.39; AIA: $14 \pm 9\%$) and EC40® (PI: 2.30 ± 0.43; AIA: $10 \pm 6\%$) were situated in between.

The Friedman test, performed on PI as well as AIA data, revealed an overall p-value < 0.001. Hence, treatment modalities were compared two by two by means of Wilcoxon's signed ranks tests. Their resulting p-values are depicted in Table 1. Mean plaque levels were significantly lower following CHX rinsing (positive control) in comparison with no therapy (negative control) ($p < 0.001$). In relation to the positive control, varnish treatment resulted in significantly higher plaque levels irrespective of the varnish that was used ($p \leqslant 0.002$). Yet, highly significant differences were also found in favour of both varnishes when compared with no therapy ($p < 0.001$). Analysis of AIA data revealed that mean plaque levels following EC40® treatment were significantly lower than those following Cervitec® treatment after 3 days of undisturbed plaque accumulation on sound enamel ($p = 0.006$).

Discussion

In this clinical study, the Quigley & Hein (1962) PI was used to assess the amount of plaque covering tooth surfaces. As such an index is based upon a quantitative assessment of dental plaque covering teeth using a non-linear, ordinal scale, small changes may be difficult to trace. Planimetric (Rekola & Scheinin 1977, Quirynen et al. 1985) and photographic techniques (Bergström 1981, Söder et al. 1993) have been proposed in the literature to quantify plaque accumulation. These techniques provide data on an interval scale, making subtle differences on plaque re-growth easier to detect, thereby increasing sensitivity. Since these methods are mostly time-consuming, they are rarely applied. Therefore, the technique of AIA (Moradi Sabzevar 1996) was used on colour slides taken from stained plaque using hand-held photography. This method, providing interval-scaled data, is proven to be highly accurate and reproducible if

Table 1. Significance testing for PI and AIA by Bonferroni-corrected Wilcoxon's signed ranks tests

Pairs of treatment modalities	PI (*p*-value)	AIA (*p*-value)
Positive control – negative control	<0.001*	<0.001*
Positive control – Cervitec®	<0.001*	<0.001*
Positive control – EC40®	0.002*	0.001*
Negative control – Cervitec®	<0.001*	<0.001*
Negative control – EC40®	<0.001*	<0.001*
Cervitec® – EC40®	0.018	0.006*

*Statistically significant at the 0.0083 level of significance. PI, plaque index; AIA, automatic image analysis.

one slide per tooth surface is made. Also, full digital image analysis systems have shown excellent reliability (Smith et al. 2001, 2004).

Given the fact that the number of subjects selected for the trial was limited, a single-blind cross-over design in which subjects acted as their own controls was chosen over a parallel design. This study design seemed even more appropriate since a distinction between ''low plaque formers'' and ''heavy plaque formers'' could be shown on the basis of our data: indeed, the lowest plaque former exhibited a mean PI of 2.43 ± 1.08 and a mean tooth surface proportion covered by plaque of $11 \pm 7\%$ in our plaque re-growth model after 3 consecutive days during which all oral hygiene measures were ceased (see negative control), whereas the heaviest plaque former showed 4.24 ± 0.98, respectively, $60 \pm 22\%$. Clearly, in spite of the fact that attempts were made to control factors likely to influence de novo plaque formation as much as possible by paying attention to eating habits and avoiding chewing gum, the data on plaque accumulation showed an extreme inter-subject diversity, stipulating the latter to be strongly host dependent. Needless to say, one would need very large samples in order to rule out this variable in a parallel-designed trial.

Looking at the results of this clinical study, it can be noted that varnish treatment resulted in significantly higher plaque levels than CHX rinsing irrespective of the varnish that was used ($p \leqslant 0.002$), implying that the latter is likely to remain the gold standard as an anti-plaque agent. Concurrently, highly significant differences were also found in favour of both varnishes when compared with no therapy ($p < 0.001$), which indicates that CHX varnish treatment is an effective means of inhibiting plaque formation in a short time span. This finding does not seem to be in accordance with Weiger et al. (1994), who could not find a plaque-inhibiting effect of Cervitec® after 3 consecutive days of undisturbed plaque formation in comparison with no treatment. This can be explained by the fact that Cervitec® was removed after 1 h of contact with the tooth surface, whereas, in the present study, the varnish was left behind for natural peeling off, thus making its contact time much longer. By choosing this approach, we acknowledge that the contact time of the varnish with the teeth might have varied substantially. Nevertheless, manufacturers' prescriptions were followed, thereby evaluating the anti-plaque effect of the varnish when it is applied as it clinically ought to be.

Although this study did not present long-term follow-up data, its results seem to be consistent with previous findings on topical CHX administration and its effect on plaque growth: indeed, Jenkins et al. (1988) reported that plaque inhibition may be achieved by topically applying CHX on enamel. Furthermore, an interesting study by Dudic et al. (1999) even indicated long-term reduction of plaque growth following application of the CHX–thymol varnish.

Analysis of AIA data revealed that Cervitec® exhibited slightly, yet significantly, higher plaque levels in comparison with EC40® following 3 days of undisturbed plaque accumulation ($p = 0.006$). PI data showed an analogue trend. The lack of statistical consolidation here might be related to the following: first, ordinal-scaled variables such as the PI are limited in showing subtle differences. Second, the sample size of a study is decisive for the power of the statistical test used: the smaller the sample size, the more difficult the statistical substantiation of a real difference in treatment efficacy becomes, especially when these differences tend to be subtle as shown on the basis of our data.

Even though it seems logical that EC40® is more effective than Cervitec® due to its high CHX concentration, one should keep in mind that this is a small-scale trial presenting preliminary data. Furthermore, both varnishes were only tested in a short time span when applied on sound enamel and not on root dentin. Indeed, submerged roots covered by overdentures impose a frequent indication for CHX varnish therapy. Here, one would expect the volatile CHX–thymol varnish to penetrate much deeper into the root dentin than EC40®, creating a CHX reservoir, possibly revealing results favouring Cervitec®. Indeed, Arends et al. (1997) published promising results with respect to the penetration capacity of Cervitec® into dentinal tubuli. As a consequence, Ekenback et al. (2000) expressed a temporal suppressive effect on *S. mutans* following Cervitec® administration on exposed sound root surfaces. However, Keltjens et al. (1992) earlier reported analogue results following EC40® administration. More research is needed comparing these varnish systems with one another in this field.

In conclusion, the data of the present study suggest that both varnishes act effectively as an anti-plaque agent within a short time span when applied on sound enamel. Moreover, EC40® was found to be more effective than Cervitec® as determined by AIA. Yet, CHX rinsing is likely to remain the therapeutic standard as an anti-plaque agent, at least on the basis of our data. We would like to emphasize that this is a preliminary investigation with no long-term clinical follow-up based on a limited study sample. Hence, the results of this trial should be interpreted with some caution, making extrapolation to similar individuals in the population impossible. Nevertheless, this study may provide a valuable onset for further research in this field leading to large-scale clinical studies with a longer observation period in order to make definitive conclusions and clinical recommendations. The impact of repeated CHX varnish administration should be addressed in future research.

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Address:
Jan Cosyn
Department of Periodontology
School of Dental Medicine
Free University of Brussels (VUB)
Laarbeeklaan 103
B-1090 Brussels
Belgium
E-mail: jan.cosyn@vub.ac.be

Clinical Relevance

Scientific rationale: The consideration that professionally applied dental varnishes overcome the non-compliance of the patient as opposed to mouth rinses makes them an appealing vehicle for CHX delivery. The microbiological impact of various varnish systems on dental plaque is well documented. However, reports regarding their anti-plaque effect on a clinical level are limited to non-existing. Therefore, a preliminary study was conducted evaluating the anti-plaque effect of two CHX varnishes – Cervitec® and EC40® – using a 3-day plaque re-growth model.

Principal findings: Both varnishes were effective in reducing de novo plaque formation. Yet, the anti-plaque effect of CHX rinsing was superior.

Practical implications: Based on these preliminary data, CHX rinsing is likely to remain the therapeutic standard as an anti-plaque agent. However, large-scale clinical studies with a longer observation period are mandatory to make definitive conclusions and clinical recommendations. The impact of repeated varnish applications should also be addressed in future trials.