

# Antibacterial Effect and Substantivity of Toothpaste Slurries In Vivo

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**Purpose:** This double-blind, clinical, cross-over study evaluated the antibacterial effect of three toothpastes (ASF, HTP and STP) and a chlorhexidine mouthrinse (0.2%; CHX; positive control) after a single application on established biofilm over a period of 24 h (substantivity).

**Materials and Methods:** Twenty-four subjects refrained from all oral hygiene measures for a period of 72 h. After 48 h, a baseline biofilm sample was taken and vitality of the biofilm flora was examined (baseline, VF<sub>0</sub>). Then they rinsed for 1 min with one of the randomly allocated, freshly prepared toothpaste slurries (ASF, HTP, STP) or CHX. Further biofilm samples were taken every second hour up to 14 h as well as 24 h after rinsing, and biofilm vitality was assessed (VF<sub>2-24</sub>). After a wash-out period of 4 days, a new test cycle was started.

**Results:** All subjects (18 female, 6 male) finished the four test cycles. At VF<sub>2</sub>, all products showed a statistically significant reduction in vitality compared to VF<sub>0</sub> ( $p < 0.05$ ). CHX and ASF revealed the most pronounced effect (49% and 40% reduction), while the other toothpastes (HTP: 24%, STP: 11%) reached lower but still statistically significant effects. At each further time point CHX and ASF showed the lowest biofilm vitality. ASF demonstrated a significant antibacterial effect on dental biofilm over a 24-h period compared to baseline and superiority over both other toothpastes at time points VF<sub>2</sub>–VF<sub>14</sub>.

**Conclusion:** ASF toothpaste showed a significant antibacterial action on biofilm and a high substantivity which was maintained up to 24 hours.

**Key words:** amine fluoride, biofilm vitality, chlorhexidine, substantivity, stannous fluoride, toothpaste

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Dental biofilm is the main cause of caries and periodontal diseases. A toothbrush used together with toothpaste is one of the most important ways in which these diseases can be prevented.<sup>11</sup> However, fluorides, which are the main ingredients in conventional toothpastes, are only effective for caries prophylaxis. Current German data have

shown that although periodontitis in younger and older adult populations is decreasing, the prevalence of moderate and severe periodontitis among young and older adults is still high (51.6% and 64.6%, respectively). The publishers themselves emphasise that due to the demographic trend, treatment needs will increase.<sup>27</sup>

Besides fluorides, which are proven responsible for caries decline, there is great interest in using active substances which can support often suboptimal mechanical plaque removal.<sup>9</sup> In view of the high prevalence of periodontitis worldwide, it was recently stated that managing gingivitis by adjunctive chemical plaque control is, i.a., an important part of periodontitis prevention.<sup>17</sup> Apart from a direct antiplaque effect, active ingredients should show high substantivity.<sup>19</sup> This term describes the adsorption and maintenance of antimicrobial activity of oral hygiene products beyond the application time.<sup>19</sup> This is necessary to prevent bacterial recolonisation until the next tooth cleaning. To yield high substantivity, active ingredients should remain at a high concentration for a long time after the application at the desired location to provide 'slow release'.<sup>8,28</sup>

Especially due to its high substantivity, chlorhexidine (CHX) is still the gold standard for antibacterial agents in dentistry,<sup>26</sup> with action against a wide array of bacteria, in-

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cluding Gram-positive and Gram-negative bacteria, dermatophytes and some lipophilic viruses.<sup>18</sup> CHX 0.2% should be used based on a therapeutic indication, and not for long-term daily mouth rinsing. However, due to its proven antibacterial efficacy, it is widely used as a positive control in clinical studies aiming at comparing the antibacterial effect of daily oral hygiene products, such as toothpastes or mouthrinses. Other well-known substances used in toothpastes are metal ions, essential oils, amine fluoride/stannous fluoride and triclosan, which have proven antibacterial effects and are recommended for long-term use.<sup>14,29,33</sup> Since other components of oral hygiene formulations could increase, decrease, mask or nullify the substantivity of the supposed active agent, the complete formulation should always be examined.<sup>1</sup>

Different test designs were used to test the substantivity of toothpaste: either after single brushing with preparations,<sup>10</sup> or after only rinsing with preparations to test the mere antibacterial effect on saliva<sup>2,3,19</sup> or established plaque.<sup>8,34</sup> This is important in so far as an active agent affects remaining established plaque which is not reached by the toothbrush, and its effect should prevent renewed establishment of plaque for as long as possible. Substantivity can be measured by the quantity of active substance remaining in the oral cavity,<sup>20,22,23</sup> the effect on salivary bacteria<sup>2,3,19</sup> or by the vital fluorescence technique, which offers qualitative information about the real activity, i.e., the antibacterial effect of the active substance available at a certain time point on the vital dental biofilm.<sup>8,10,34</sup>

The aim of this study was to assess the antibacterial effect and substantivity of commercially available toothpastes applied as slurries on established dental biofilms over 24 h by measuring the vitality of dental biofilm at different time points.

## MATERIALS AND METHODS

The clinical study was randomised, double-blind, controlled, cross-over, monocentric and comprising 24 subjects, with a previously described design.<sup>8</sup> It was performed in the Department of Periodontology of the Philipps University, Marburg. The protocol for the study was reviewed and approved by the Medical Ethics Committee of Philipps University, Marburg, and not commenced until approval had been obtained (#244/12). The study was performed in compliance with Good Clinical Practice (GCP) and the Declaration of Helsinki. Subjects were only included into the study after having given written consent.

### Study Population

An initial screening ascertained the eligibility of the subjects to take part in the study. Of the 32 volunteers screened, 8 did not meet the in- or exclusion criteria. After written patient information was provided, a total of 24 (18 female and 6 male) subjects with a mean age of  $25.7 \pm 7.4$  years were included in the study and signed a declaration of consent (see study flowchart, Fig 1).

Inclusion criteria were good health, at least 20 natural and assessable teeth, and effective contraception. Exclusion

criteria were on-going dental treatment or any other medical treatment of the oral cavity, any known allergy to previously used oral hygiene products or dental materials which are used in the oral cavity or in the throat, any known allergy to the ingredients of the study product or the standard toothpaste used during the study, current periodontitis or non-physiological tooth mobility, any pathological change of the oral mucosa or gingiva, poor oral hygiene (papilla bleeding index >30%), use of antibiotics or other medicines during the last six months which could disturb plaque formation, abuse of drugs and alcohol, pregnancy or breastfeeding, and the participation in a clinical study within the previous 30 days.

### Test Products

The following toothpastes and solutions were tested:

- ASF: toothpaste with amine fluoride/stannous fluoride, 1400 ppm fluoride (Meridol toothpaste, Colgate-Palmolive Europe; Therwil, Switzerland)
- HTP: toothpaste with herbal ingredients and 1400 ppm (sodium) fluoride (Parodontax mit Fluorid, GlaxoSmith-Kline; Bühl, Germany)
- CHX: Chlorhexamed forte 0.2% mouthrinse (with alcohol; GlaxoSmith-Kline)
- STP: (standard) toothpaste with 1450 ppm (sodium) fluoride (Signal Kariesschutz, Unilever Deutschland; Hamburg, Germany)

### Randomisation and Blinded Supply of the Products

To prevent uncontrollable influences, the study was performed randomly and under triple blind conditions: neither the subjects, nor the investigator, nor the statistician knew which product was tested. All products were delivered by the sponsor in identical tubes and supplied by a laboratory assistant, who was not otherwise involved in the study. The subjects received the allocated toothpaste slurry or rinse (in case of CHX) in the same cup, not being aware of the preparation. The subjects were randomly allocated to the different treatments according to a random allocation table and assigned to numbers by the order of their attendance for the initial treatment phase according to a randomisation scheme provided by the sponsor. The randomisation code was kept in a sealed envelope and was disclosed after all examinations were finished.

### Study Design

Recruitment and study schedule are illustrated in the flowchart (Fig 1). In order to standardise oral conditions and create similar initial conditions, all participants had to undergo professional tooth cleaning, which was performed before the start of the study and removed all tartar and calculus at the start of the study. Each subject was given a toothbrush (elmex interX soft toothbrush, Colgate-Palmolive Europe) and a toothpaste with a low concentration of active substances (Signal caries protection, Unilever, Hamburg, Germany) to use for their oral hygiene homecare routine during the wash-in phase at the start of the study and the wash-out phases between the individual cycles. Afterwards, the subjects were required to dispense with their oral hygiene homecare rou-

**Fig 1** Flow chart of the study.

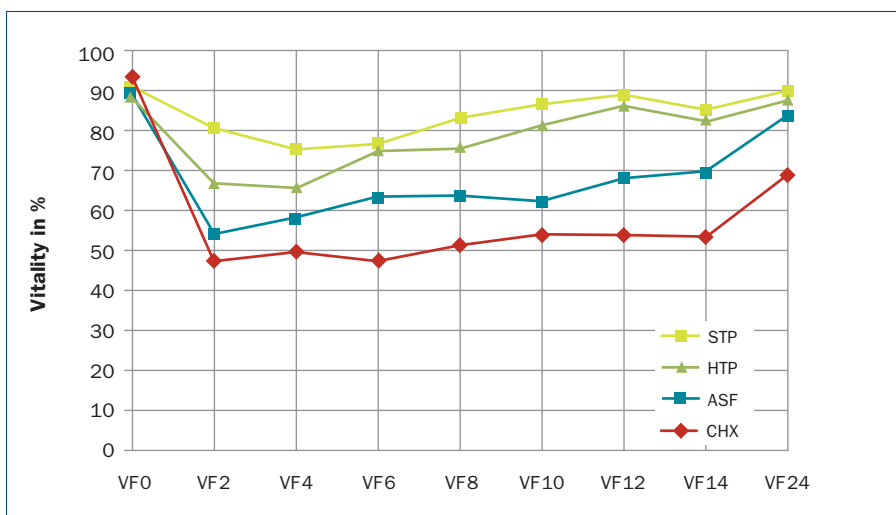


tine for the next 72 h. After 48 h of unhindered plaque formation, a dental probe was used to obtain a plaque sample (48-h-old plaque biofilm) taken from the vestibular and oral aspects of two specific teeth.<sup>16,36</sup> The sample was applied to a microscope slide, stained and analysed under the microscope by a vital fluorescence technique to determine a baseline vitality value (VF<sub>0</sub>). This technique was recently reviewed and described in detail.<sup>32</sup> After the first sample was taken, the participants were required to rinse for 1 min with a randomly allocated solution (10 ml) or slurry under the supervision of a lab assistant not otherwise involved in the study. The slurries were mixed from 3 g toothpaste and 10 ml distilled water into a homogenous paste, immediately

filled into cups and distributed to the volunteers according to the randomisation schedule. The toothpastes were prepared as a slurry to prevent any mechanical oral hygiene action from having an effect on the plaque biofilm.

Further plaque samples were taken 2, 4, 6, 8, 10, 12, 14 and 24 h after VF<sub>0</sub> from two teeth each. All of these samples were stained and analysed as described before (VF<sub>2</sub>–VF<sub>24</sub>).

Adverse events were recorded at every visit. Furthermore, the volunteers answered a questionnaire about their acceptance and also possible side effects of the toothpastes at the end of each test cycle, recorded in a quality-of-life questionnaire.



**Fig 2** Biofilm vitality (in %) before (VF<sub>0</sub>) and after application of the products at the different time points (VF<sub>2</sub>–VF<sub>24</sub>).

A new test cycle was started after a wash-out period of 4 days, in which the volunteers could return to their usual oral hygiene routine. This period was needed to avoid carry-over effects. During these phases, the participants had to use the toothpaste and the toothbrush provided.

### Microbiological Evaluation: Biofilm Vitality (VF%) as the Primary Outcome Measure

The vital fluorescence technique – in which vital bacterial cells are stained green by fluorescein diacetate (FDA) and metabolically inactive (nonvital) cells are stained red by ethidium bromide (EB) – was recently extensively described and is recognised as a valid method.<sup>32</sup> After the completion of the staining reaction, a cover slip (Menzel; Braunschweig, Germany) was pressed firmly down onto the sample. The evaluation was performed under a microscope (Axio Imager A2, Carl Zeiss; Göttingen, Germany). Four pictures of different parts of each sample were taken with a digital camera (AxioCam; Carl Zeiss). The vitality of the plaque samples was determined using an image analysis programme (Axion Vision Rel. 4.8) to differentiate green (vital) and red (nonvital) bacteria. The vitality (VF percentage) was determined by the proportion of vital cells to the sum of vital and nonvital cells of the sample.<sup>8</sup>

### Further Outcome Measures: Quality of Life Questionnaire

After the last plaque sample had been taken for each product, the participants were given a questionnaire to complete (quality of life). In this way, they were able to provide information on flavour, taste disturbance and mouth burning during the application of the products, and also mention any possible side effects or irritation.

### Sample Size Estimate, Power Calculation and Statistical Analysis

Based upon mean outcomes ( $\pm$  SD) of a previous study,<sup>8</sup> for a two-sided test of equality of means at the 0.05 level of significance with 80% power, a need of  $n = 21$  volunteers

was calculated. With regard to possible dropouts, a sample size of  $n = 24$  was determined.

Data from this clinical trial were documented on report forms and the results of VF entered into an Excel table and then into SPSS (v. 21). Statistical analysis was carried out by a statistician (CH). Besides the descriptive analysis (mean, standard deviation, box plots, line diagrams), the results of each of the four treatment groups at each measurement time (2, 4, 6, 8, 10, 12, 14, 24 h) were compared with baseline (0 h). As these were observations on the same test subject, the paired Wilcoxon signed-rank test was carried out for intragroup comparisons. A repeated measures linear mixed model was calculated to examine the intergroup differences separately at each time of measurement. The cross-over design of the study was taken into account, and therefore four values (corresponding to the four groups) were measured each time on the same test subject.

### Monitoring

The study was monitored in accordance with international ethics and scientific quality standards (Good Clinical Practice directive) by RMC Consult; Rheinfelden, Germany.

## RESULTS

Each of the 24 participants (18 female and 6 male) between the ages 19 and 48 years (mean age 25.7 years) completed all test cycles. Figure 2 illustrates the course of substantivity during the 24-h observation period. Table 1 gives all the vitality values  $\pm$  standard deviations as well as the results of comparisons with baseline values (VF<sub>0</sub>) for the four groups. Table 2 lists the statistical comparisons between the products at each follow-up.

Two hours after application, all toothpastes caused a significant reduction in plaque vitality (VF<sub>2</sub>) when compared to their corresponding baseline value (VF<sub>0</sub>). CHX and ASF demonstrated the greatest reduction (49% and 40%, re-

**Table 1 Vitality values (VF in %; ± SD) at baseline (VF<sub>0</sub>) and at different time points (VF<sub>2</sub>–VF<sub>24</sub>) as well as statistical comparisons compared to baseline**

	VF <sub>0</sub>	VF <sub>2</sub>	P VF <sub>2</sub> vs VF <sub>0</sub>	VF <sub>4</sub>	P VF <sub>4</sub> vs VF <sub>0</sub>	VF <sub>6</sub>	P VF <sub>6</sub> vs VF <sub>0</sub>	VF <sub>8</sub>	P VF <sub>8</sub> vs VF <sub>0</sub>	VF <sub>10</sub>	P VF <sub>10</sub> vs VF <sub>0</sub>	VF <sub>12</sub>	P VF <sub>12</sub> vs VF <sub>0</sub>	VF <sub>14</sub>	P VF <sub>14</sub> vs VF <sub>0</sub>	VF <sub>24</sub>	P VF <sub>24</sub> vs VF <sub>0</sub>
CHX	93.8 ± 3.7	47.7 ± 12.3	<0.001 ***	49.9 ± 14.9	<0.001 ***	47.6 ± 17.1	<0.001 ***	51.5 ± 14.1	<0.001 ***	54.1 ± 17.9	<0.001 ***	53.8 ± 18.1	<0.001 ***	54.1 ± 21.5	<0.001 ***	69.4 ± 20.5	<0.001 ***
ASF	90.3 ± 7.6	54.6 ± 12.1	<0.001 ***	58.3 ± 14.7	<0.001 ***	63.3 ± 13.3	<0.001 ***	63.9 ± 16.6	<0.001 ***	62.6 ± 15.8	<0.001 ***	68.6 ± 14.2	<0.001 ***	70.1 ± 12.0	<0.001 ***	84.2 ± 8.0	0.006 **
HTP	89.1 ± 7.6	67.4 ± 13.1	<0.001 ***	66.3 ± 15.5	<0.001 ***	75.3 ± 12.8	<0.001 ***	75.9 ± 13.0	0.002 **	81.8 ± 11.5	0.052 n.s.	86.2 ± 8.9	0.199 n.s.	83.4 ± 9.4	0.049 *	88.3 ± 5.3	0.648 n.s.
STP	90.8 ± 6.5	81.0 ± 9.7	0.001 ***	75.6 ± 14.6	<0.001 ***	76.6 ± 18.3	0.001 ***	83.6 ± 9.2	0.015 *	86.6 ± 9.1	0.130 n.s.	89.2 ± 7.8	0.587 n.s.	85.0 ± 10.5	0.670 n.s.	90.7 ± 5.9	0.819 n.s.

n.s.: not significant, shaded in blue; \*p ≤ 0.05; \*\*p ≤ 0.01; \*\*\*p ≤ 0.001, Wilcoxon Rank-Sum test.

**Table 2 Statistical comparison between the groups at the different time points (VF<sub>0</sub>–VF<sub>24</sub>)**

	pVF <sub>0</sub>	pVF <sub>2</sub>	pVF <sub>4</sub>	pVF <sub>6</sub>	pVF <sub>8</sub>	pVF <sub>10</sub>	pVF <sub>12</sub>	pVF <sub>14</sub>	pVF <sub>24</sub>
CHX vs ASF	0.479 n.s.	0.229 n.s.	0.278 n.s.	0.002 **	0.013 *	0.241 n.s.	0.004 **	0.001 ***	0.001 ***
CHX vs HTP	0.078 n.s.	0.000 ***	0.001 ***	0.000 ***	0.000 ***	0.000 ***	0.000 ***	0.000 ***	0.000 ***
CHX vs STP	0.669 n.s.	0.000 ***	0.000 ***	0.000 ***	0.000 ***	0.000 ***	0.000 ***	0.000 ***	0.000 ***
ASF vs HTP	1.000 n.s.	0.001 ***	0.335 n.s.	0.037 *	0.018 *	0.000 ***	0.000 ***	0.009 **	1.000 n.s.
ASF vs STP	1.000 n.s.	0.000 ***	0.001 ***	0.023 *	0.000 ***	0.000 ***	0.000 ***	0.003 **	0.339 n.s.
HTP vs STP	1.000 n.s.	0.001 ***	0.159 n.s.	1.000 n.s.	0.305 n.s.	1.000 n.s.	1.000 n.s.	1.000 n.s.	1.000 n.s.

n.s.: not significant; \*p ≤ 0.05; \*\*p ≤ 0.01; \*\*\*p ≤ 0.001 by repeated measures linear mixed models.

spectively) compared to VF<sub>0</sub>. HTP and STP resulted in lower reductions of 24% and 11%, respectively, but still had significant effects (p < 0.05).

At VF<sub>2</sub>, CHX and ASF were significantly different from PAR (p ≤ 0.001), while at the same time point, all three products were significantly different from STP. The VF values of ASF and CHX were not significantly different from each other at VF<sub>2</sub>.

Over the 24-h time period, CHX demonstrated significantly lower vitality values after every application (VF<sub>2</sub>–VF<sub>24</sub>) compared to HTP and STP. CHX was significantly different from ASF at VF<sub>6</sub>, VF<sub>8</sub>, VF<sub>12</sub>, VF<sub>14</sub> and VF<sub>24</sub>, while the values were not significantly different at times VF<sub>2</sub>, VF<sub>4</sub> and VF<sub>10</sub>.

When compared to HTP, the use of ASF showed significantly lower vitality values at all time points except VF<sub>4</sub> and VF<sub>24</sub>.

### Evaluation of the Quality of Life Questionnaire

The questionnaire was evaluated descriptively and the results are shown in Table 3. Overall, ASF and STP were rated

the best, with similar assessments. Regarding the taste of the product, 19 out of the 24 test subjects found ASF to have a pleasant taste, while 20 of 24 thought STP tasted pleasant. Three participants described ASF as mild, four said the same of STP. One subject found ASF tasted spicy and another one described it as bitter. While 10 subjects found CHX pleasant, two thought it was unpleasant and 12 described it as spicy; a clear majority (16 of 24 test subjects) thought HTP was unpleasant. One subject in each case found HTP spicy or bitter, while three thought it was salty. Only three test subjects found HTP to be pleasant. None of the participants noticed any unusual taste after using STP, but one did after ASF, two after HTP, and nine with CHX (each time out of 24). The feeling of freshness experienced after use was rated on a visual analogue scale of 0 to 10 (0 = not fresh at all; 10 = very fresh). In this assessment, ASF ranked first, with a mean score of 7.4, better than CHX with 7.2. The corresponding scores for STP and HTP were 6.3 and 4.9, respectively. No subject had an unpleasant sensation after rinsing with STP, although two

**Table 3 Evaluation of the quality of life questionnaire**

	ASF	HTP	STP	CHX	
Taste of the product	pleasant	19	3	20	10
	unpleasant	0	16	0	2
	spicy	1	1	0	12
	mild	3	0	4	0
	bitter	1	1	0	0
	others	0	3 salty	0	0
Unusual taste (irritation)	yes: 1 no: 23	yes: 2 no: 22	yes: 9 no: 15	yes: 0 no: 24	
Feeling of freshness after use (VAS 0 = not fresh at all, 10 = very fresh)	7.4	4.9	7.2	6.3	
Unpleasant sensation after rinsing	yes: 2 no: 22	yes: 3 no: 21	yes: 4 no: 20	yes: 0 no: 24	
Would you use the product regularly?	yes: 16 no: 8	yes: 2 no: 22	yes: 12 no: 12	yes: 17 no: 7	

test subjects did after ASF, three after HTP, and four after CHX (each time out of 24). Asked whether they would use the product regularly, 17 said they would use STP and 16 agreed they would use ASF. Half of the participants (12/24) would use CHX regularly but only two said they could see themselves using HTP on a regular basis.

## DISCUSSION

Aside from a mere antibacterial effect, active agents in oral hygiene products need a prolonged effect far beyond the single rinsing time; this is called substantivity and clearly differs from requirements for (antiseptic) agents generally used in medicine. Gaffar et al<sup>21</sup> stressed that retention of an active substance per se is not the only factor for 'anti-plaque activity'; more importantly, the remaining concentration must remain biologically active. This was confirmed in the 2nd European Workshop on Periodontology.<sup>28</sup>

The aim of this study was to examine the antibacterial effect as well as the substantivity independently of their mechanical cleaning properties. Many studies address this issue.<sup>1,4,8,12,24,25,34</sup> However, all authors point out that parameters for measuring a biologically active substance are limited. Due to ethical concerns, radiolabelling of substances with <sup>14</sup>C – as performed with chlorhexidine<sup>13</sup> – is no longer done. While different investigations only measure the quantity of active substance remaining in the oral cavity,<sup>20,22,23</sup> the vital fluorescence technique offers a qualitative statement about their real activity and clinical effect, i.e. the antibacterial effect on the vital dental biofilm of the active substance available at a certain time point.<sup>8,32,34</sup>

To avoid overlaps or interactions with the mechanical cleaning effect by toothbrushing but to simultaneously

mimic the clinical situation, toothpastes were used as slurries by mixing with water. The ratio was chosen so that simple rinsing reproduces the quantity of the active substances present in the oral cavity during normal toothbrushing.<sup>1,4,8,12,24,25</sup> While some studies used water as a negative control and not a placebo paste due to the complexity of toothpastes and the possible effects of common ingredients, e.g. detergents,<sup>1,4</sup> the present study examined the clinical effect of commercially available toothpastes. Therefore, three toothpastes with a known antibacterial agent (ASF) or herbal ingredients (HTP) and a standard toothpaste with not specifically declared antibacterial agents (STP) were used. A chlorhexidine solution served as a positive control, since it is still the gold standard<sup>26</sup> and allows the comparison with previous studies.

Aside from CHX, ASF toothpaste yielded the lowest vitality at every time point within the present study and demonstrated a significant antibacterial effect on the dental biofilm over a 24-h period. Compared to the other toothpastes, the effect of ASF and CHX two hours after rinsing was considerably higher. The mere antibacterial effect of amine fluoride/stannous fluoride and its influence on plaque regrowth and gingivitis has already been established in different clinical studies.<sup>6,7,9,15,16</sup> The present study also confirmed the superior effect of CHX, followed by ASF, compared to essential oils or other agents against plaque and gingivitis, as also found in previous studies.<sup>6,7,15,16</sup> Those studies only speculated that different substantivity could be the reason for an antibacterial effect, since ASF started at a comparable level with CHX, but declined during the course of the study, while other agents in toothpastes could not reach the effect of CHX and ASF.

The literature contains no data on the substantivity of the HTP toothpaste or its combination of ingredients, which

is based on herbal extracts such as myrrh, echinacea, chamomile and rhatany, as well as bicarbonate. In other clinical studies, no specific effect on biofilm vitality or on clinical parameters could be found.<sup>30,31</sup> The present data demonstrate a significant reduction of vital plaque biofilm flora from time point VF<sub>2</sub> to VF<sub>8</sub> and again at VF<sub>14</sub> compared to the baseline (VF<sub>0</sub>). This suggests a good substantivity of the HTP, albeit not reaching that of CHX and ASF. The same is true for the STP, which served – in the broadest sense – as control, since it contains no specifically antibacterial agents. This toothpaste also yielded a significant antibacterial effect up to VF<sub>8</sub> compared to its baseline, which then however diminished and was significantly lower than CHX and ASF.

## CONCLUSION

CHX as well as all toothpastes showed significant antibacterial effects until 8 h after application compared to their baseline values. The effect of CHX and ASF was continuously present over 24 h. while the effect of both other products (HTP and STP) diminished and was no longer significant. The participants' subjective assessment of the products rated ASF and STP similarly, both products being viewed much more positively than the other two.

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## REFERENCES

1. Addy M, Jenkins S, Newcombe R. The effect of triclosan, stannous fluoride and chlorhexidine product on: (I) Plaque regrowth over a 4-day period. *J Clin Periodontol* 1990;17:693–697.
2. Addy M, Jenkins S, Newcombe R. The effect of some chlorhexidine-containing mouthrinses on salivary bacterial counts. *J Clin Periodontol* 1991;18:90–93.
3. Addy M, Greenman J, Renton-Harper P, Newcombe R, Doherty F. Studies on stannous fluoride toothpaste and gel (2). Effects on salivary bacterial counts and plaque regrowth in vivo. *J Clin Periodontol* 1997;24:86–91.
4. Addy M, Willis L, Moran J. Effect of toothpaste rinses compared with chlorhexidine on plaque formation during a 4-day period. *J Clin Periodontol* 1983;10:89–99.
5. Addy M, Jenkins S, Newcombe R. The effect of triclosan, stannous fluoride and chlorhexidine product on: (I) Plaque regrowth over a 4-day period. *J Clin Periodontol* 1990;17:693–697.
6. Arweiler NB, Netuschil L, Reich E. Alcohol-free mouthrinse solutions to reduce supragingival plaque regrowth and biofilm vitality. *J Clin Periodontol* 2001;28:168–174.
7. Arweiler NB, Reich E, Brex M, Dörner M, Netuschil L. Influence of ethanol on the antibacterial and anti-plaque efficacy of an amine fluoride/stannous fluoride mouthrinse solution. *J Parodontol Implantol Orale* 2001;20:331–340.
8. Arweiler NB, Auschill TM, Reich E, Netuschil L. Substantivity of toothpaste slurries and their effect on reestablishment of the dental biofilm. *J Clin Periodontol* 2002;29:615–621.
9. Auschill TM, Hein N, Hellwig E, Follo M, Sculean A, Arweiler NB. Effect of two antimicrobial agents on early in situ biofilm formation. *J Clin Periodontol* 2005;32:147–152.
10. Auschill TM, Deimling D, Hellwig E, Arweiler NB. Antibacterial effect of two toothpastes following a single brushing. *Oral Health Prev Dent* 2007;5:25–32.
11. Axellson P, Lindhe J, Nyström B. On the prevention of caries and periodontal disease. Results of a 15-year longitudinal study in adults. *J Clin Periodontol* 1991;18:182–189.
12. Binney A, Addy M, McKeown S, Everatt L. The choice of controls in toothpaste studies. The effect of a number of commercially available toothpastes compared to water on 4-day plaque regrowth. *J Clin Periodontol* 1996;23:456–459.
13. Bonesvoll P, Lökken P, Rølla G, Paus PN. Retention of chlorhexidine in the human oral cavity after mouth rinses. *Arch Oral Biol* 1974;19:209–212.
14. Brex M. Strategies and agents in supragingival chemical plaque control. *Periodontology* 2000 1997;15:100–108.
15. Brex M, Netuschil L, Reichert B, Schreil G. Efficacy of Listerine®, Meridol® and chlorhexidine mouthrinses on plaque, gingivitis and plaque bacteria vitality. *J Clin Periodontol* 1990;17:292–297.
16. Brex M, Brownstone E, Mac Donald L, Gelskey S, Cheang M. Efficacy of Listerine, Meridol and Chlorhexidine mouthrinses as supplements to regular tooth cleaning measures. *J Clin Periodontol* 1992;19:202–207.
17. Chapple IL, Van der Weijden F, Doerfer C, Herrera D, Shapira L, Polak D, et al. Primary prevention of periodontitis: managing gingivitis. *J Clin Periodontol* 2015;42(suppl 16):S71–76.
18. Denton GW. Chlorhexidine. In: Block SS (ed). *Desinfection, sterilization and preservation*. Philadelphia: Lea and Febiger 1991;274–289.
19. Elworthy A, Greenman J, Doherty FM, Newcombe RG, Addy M. The substantivity of a number of oral hygiene products determines by the duration of effects on salivary bacteria. *J Periodontol* 1996;67:572–576.
20. Gaffar A, Afflito J, Nabi N, Herles S, Kruger I, Olsen S. Recent advances in plaque, gingivitis, tartar and caries prevention technology. *Int Dent J* 1994;44:63–71.
21. Gaffar A, Afflito J, Nabi N. Chemical agents for the control of plaque and microflora: an overview. *Eur J Oral Sci* 1997;105:502–507.
22. Günbay S, Bicakci N, Parlak H, Guneri T, Kirilmaz L. The effect of zinc chloride dentifrices on plaque growth and oral zinc levels. *Quint Int* 1992;23:619–624.
23. Harrap GJ, Best JS, Saxton CA. Human oral retention of zinc from mouthwashes containing zinc salts and its relevance to dental plaque control. *Arch Oral Biol* 1984;29:87–91.
24. Jenkins S, Addy M, Newcombe R. Triclosan and sodium lauryl sulphate mouthrinses (II). Effects of 4-day plaque regrowth. *J Clin Periodontol* 1991;18:145–148.
25. Jenkins S, Addy M, Newcombe R. Comparison of cetylpyridinium chloride, triclosan and chlorhexidine mouthrinse formulations for effects on plaque regrowth. *J Clin Periodontol* 1994;21:441–444.
26. Jones CG. Chlorhexidine: is it still the goldstandard? *Periodontology* 2000 1997;15:55–62.
27. Jordan AR, Micheelis W. Fünfte Deutsche Mundgesundheitsstudie (DMS V). Materialienreihe Bd. 35, Institut der Deutschen Zahnärzte (Hrsg.), Köln: Deutscher Zahnärzte Verlag DÄV, 2016.
28. Lang NP, Newman HN. Consensus report of session II. In: Lang NP, Kar-ring T, Lindhe J (eds). *Proceedings of the 2nd European Workshop on Periodontology*. Chicago: Quintessence 1997;192.
29. Mandel ID. Chemotherapeutic agents for controlling plaque and gingivitis. *J Clin Periodontol* 1988;15:488–498.
30. Moran J, Addy M, Newcombe R. Comparison of an herbal toothpaste with a fluoride toothpaste on plaque and gingivitis. *Clin Prev Dent* 1991;13:12–15.
31. Mullally BH, James JA, Coulter WA, Linden GJ. The efficacy of herbal-based toothpaste on the control of plaque and gingivitis. *J Clin Periodontol* 1995;22:686–689.
32. Netuschil L, Auschill TM, Sculean A, Arweiler NB. Confusion over live/dead stainings for the detection of vital microorganisms in oral biofilms-which stain is suitable? *BMC Oral Health* 2014;11:14.
33. Sanz M, Serrano J, Iniesta M, Santa Cruz I, Herrera D. Antiplaque and anti-gingivitis toothpastes. *Monogr Oral Sci* 2013;23:27–44.
34. Weiland B, Netuschil L, Hoffmann T, Lorenz K. Substantivity of amine fluoride/stannous fluoride following different modes of application: a randomized, investigator-blind, placebo-controlled trial. *Acta Odontol Scand* 2008;66:307–313.