In-vitro activity of sodium-hypochlorite gel on bacteria associated with periodontitis

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Objectives: The aim of the present study was to assess the antimicrobial activity of a sodium hypochlorite formulation incl. its components against bacteria associated with periodontal disease.

Materials and Methods: Sodium hypochlorite formulation (NaOCl gel), its components sodium hypochlorite (NaOCl) and the activating vehicle were compared with 0.1% chlorhexidine digluconate (CHX) solution. The antimicrobial activity was proven by determination of minimal inhibitory concentrations (MIC), minimal bactericidal concentrations, and killing assays. Furthermore, the influence on formation as well as on a 4-days old 6-species biofilm was tested.

Results: Except for one strain (*Parvimonas micra* ATCC 33270 in case of NaOCl gel) the MICs both of the CHX solution and NaOCl gel did not exceed 10% of the formulations' concentration. In general MICs of the NaOCl gel were equal as of the CHX solution against Gram-negatives but higher against Gram-positive bacteria. CHX but not NaOCl gel clearly inhibited biofilm formation, however the activity of NaOCl gel was more remarkable on a 4-d old biofilm. NaOCl killed bacteria in the biofilm and interfered with the matrix.

Conclusions: The NaOCl gel acts antimicrobial in particular against Gram-negative species associated with periodontitis. Moreover, its component NaOCl hypochlorite is able to alter biofilm matrices.

Clinical relevance: The NaOCl gel may represent a potential alternative for adjunctive topical antimicrobial treatment in periodontitis

Key words: sodium hypochlorite, periodontal infection, biofilm

1. Introduction

Periodontitis is a chronic inflammatory disease of the tooth supporting tissues associated with high counts of certain bacterial species interacting with the host' immune system [1]. Oral microbial-plaque communities are biofilms composed of numerous bacteria on host surfaces. [2]. Bacteria more present in patients with chronic periodontitis than in periodontally healthy subjects are *Treponema denticola*, *Porphyromonas gingivalis*, *Tannerella forsythia*, and several others [3]. *P. gingivalis* a Gram-negative anaerobe bacterium, is considered a key-stone pathogen in developing periodontal disease [4].

Non-surgical mechanical removal of the hard and soft microbial deposits from the root surfaces (i.e. scaling and root planing (SRP)) is the standard in any cause-related periodontal therapy [5]. Substantial evidence indicates that during supportive periodontal therapy (SPT) periodontitis can be successfully treated and controlled by thorough mechanical plaque removal by the patient coupled with supra -and subgingival debridement by the therapist with or without the use of local antimicrobials [6]. During the last decade, various antimicrobials such as chlorhexidine, azithromycin, metronidazole, doxycycline, minocycline and tetracycline used subgingivally in conjunction with SRP have been tested [7]. Among the supragingivally used antiseptics, chlorhexidine based dentifrices/gels are still being the gold standard although tooth surface discoloration is observed as side effect [8].

An alternative approach to improve the outcomes of subgingival SRP might be the application of sodium hypochlorite. Its broad antimicrobial activity, fast bactericidal action and non-toxicity at application concentration, has been known since many years [9]. Already in 1918 it was published that sodium hypochlorite has a higher dissolving effect on necrotic tissue than on vital one [10]. However its use on everyday basis in dentistry is known basically in endodontics as one of the main canal irrigants [11]. Activity of sodium hypochlorite is depending on pH. It is most active when applied at neutral or slightly acidic pH [12]. Comparison of the activity of different antiseptics on experimental biofilm with various endodontic/periodontal pathogens, showed that the highest bactericidal activity was obtained with 2.25% sodium hypochlorite and 10% povidone-iodine and last by 0.2% chlorhexidine [13]. Already in the early 80ies of the last century , the use of sodium hypochlorite combined with curretage was histologically shown to be effective in reducing soft tissue inflammation in SPT [14]. The results showed that sodium hypochlorite achieves predictable chemolysis of the soft tissue wall of the periodontal pocket with minimal effect on the adjacent tissues while the

antiseptic did not impede the healing phase [14]. Moreover, it was suggested by Perova [15], that the use of 0.1% sodium hypochlorite during periodontal surgeries might improve the healing, through a markedly better regeneration of the connective tissue at the gingival base of the sites. Despite its promising properties, sodium hypochlorite did not stay in the light of interest for long, until it has been rediscovered just recently. An oral mouthrinse with 0.05% sodium hypochlorite resulted in significant reduction in supragingival biofilm accumulation and gingival inflammation [16]. In subsequent studies twice-weekly rinsing with 0.25% sodium hypochlorite solution decreased dental plaque level and reduced bleeding on probing in periodontal pockets [17, 18].

A formulation of a sodium hypochlorite gel to be used in periodontal therapy has been recently introduced to the market. It is composed of two components, sodium hypochlorite solution and a mixture of amino acids. After combining the two substrates different chloramines are synthesized from free sodium hypochlorite, which may additionally enhance the antimicrobial activity.

The purpose of this in vitro study was to determine the antimicrobial activity of the sodium hypochlorite gel and its components in comparison to chlorhexidine digluconate on microbial species associated with periodontitis including *P. gingivalis, T. forsythia, Aggregatibacter actinomycetemcomitans, Parvimonas micra* and others.

The hypothesis was that there is a minimal inhibitory concentration of sodium hypochlorite gel, which inhibits predictably the growth and biofilm formation of bacteria associated with periodontal disease.

2. Material and methods

2.1. Substances

Test substances were component 1 (NaOCl: sodium hypochlorite solution 0.95%), component 2 (activating vehicle: glutamic acid, leucine, lysine, carboxymethyl cellulose, and ultrapure water) and sodium hypochlorite gel (NaOCl gel (Perisolv, Regedent AG, Zurich, Switzerland)), composed of the previous two components mixed together. Chlorhexidine digluconate solution

(CHX) in the concentration of 0.1% was used as a positive control, whereas 0.9% sodium chloride (NaCl) as a negative control.

2.2. Microorganisms

The following bacterial strains were tested as single bacterial species: *P. gingivalis* ATCC 33277, *T. forsythia* ATCC 43037, *Fusobacterium nucleatum* ATCC 25586, *Streptococcus gordonii* ATCC 10558, *Actinomyces naeslundii* ATCC 12104, *Parvimonas micra* ATCC 33270, *Prevotella intermedia* ATCC 25611, *A. actinomycetemcomitans* ATCC 33384, *Campylobacter rectus* ATCC 33238, *Eikenella corrodens* ATCC 23834, *Filifactor alocis* ATCC 33099, *Capnocytophaga gingivalis* ATCC 33624, *Eubacterium nodatum* ATCC 33270 and three clinical isolates of *P. gingivalis* and *T. forsythia*. The mixed microbiota consisted of the following bacterial strains: *P. gingivalis* ATCC 33277, *T. forsythia* ATCC 43037, *F. nucleatum* ATCC 25586, *S. gordonii* ATCC 10558, *A. naeslundii* ATCC 12104 and *P. micra* ATCC 33270. Before an experiment, all strains were precultivated on Schaedler agar plates (Oxoid, Basingstoke, UK) with 5% sheep blood and vitamin K addition, in an anaerobic atmosphere or with 5% CO_2 (*A. actinomycetemcomitans* ATCC 33384 and *S. gordonii* ATCC 10558).

2.3. Susceptibility tests: Determination of the minimal inhibitory concentrations and minimal bactericidal concentrations

Determination of MICs was performed by the micro-broth dilution technique using the 96-well-microtiter plates. The MICs of component 1, component 2, Perisolv and 0.1% chlorhexidine solution for single microorganisms and mixed species were checked.

After subcultivation of bacterial strains, a defined inoculum, with an adjusted turbidity of McFarland 4 (0.5 for *S. gordonii*) was added to Wilkins Chalgren broth (Oxoid) supplemented with nicotinamide adenine dinucleotide and N-acetyl muramic acid, in a 1:17 ratio. Defined concentrations of NaOCl gel, its components and chlorhexidine as positive control were added.

After 42 h of incubation time (18 h for *S. gordonii*), the growth of microorganisms was analyzed visually by checking the turbidity. MIC represents the lowest concentration without visible turbidity of the broth.

For determination of the minimal bactericidal concentration, non-turbid cultures were subcultivated on agar plates without the addition of any antimicrobial agent. After incubating, the MBC was the lowest concentration without any growth of the colonies on the agar plates (equivalent to a reduction by 99.9% of the initial inoculum).

Tests were performed in independent replicates.

2.4. Killing

A defined inoculum of microorganisms (about 10^6 /ml), prepared in doubled concentrated nutrient media (Wilkins Chalgren broth), was added to NaOCl gel in final concentrations of 20%, 10% and 5%, as well as 0.01% chlorhexidine. In this experiment the following bacterial species were used: *P. gingivalis* ATCC 3327, *T. forsythia* ATCC 43037, *P. micra* ATCC 33270 and the mixed species. After 1 h, 2 h, 6 h as well as 24 h of incubation, the numbers of viable bacteria were determined by enumeration of colony forming units (cfu). The test was performed in independent replicates.

2.5. Activity against bacteria in biofilms

In these experiments a multispecies biofilm consisting of *S. gordonii* ATCC 10558, *P. gingivalis* ATCC 33277, *T. forsythia* ATCC 43037, *F. nucleatum* ATCC 25586, *A. naeslundii* ATCC 12104 and *P. micra* ATCC 33270 was used. The influence of the test substances on the developing biofilm was examined. First the wells of 96-well-plates were covered with 20 µl of test substance. After 1 h of incubation, 10 µl of protein solution consisting of 25% serum and 5% albumin was added to the surface, followed by 170 µl of bacterial suspension. Bacterial suspension was prepared by adding a defined inoculum (1 part *S. gordonii*, 2 parts *A. naeslundii* and each 4 parts of the other species; each McFarland 4) to Wilkins Chalgren broth (Oxoid) supplemented with nicotinamide adenine dinucleotide and N-acetyl muramic acid, in a 1:17

ratio. The 96-well-plates were incubated anaerobically at 37° under static conditions. The cfu were counted after 6 h, 24 h and 48 h development of biofilm.

Additionally, the influence of the test substances was evaluated on a formed biofilm. Multispecies biofilm composed of six bacterial species was developed for four days. First the wells of 96-well-plates were covered with 10 µl of 25% v/v inactivated human serum/well for 1 h. Then bacterial suspension was prepared and mixed with the Wilkins Chalgren broth as described above. The plates were incubated in the anaerobic atmosphere at 37°C. After 48 h the medium was carefully exchanged with a new bacterial suspension of *P. gingivalis* ATCC 33277 and *T. forsythia* ATCC 43037 added to the nutrient medium before application to the wells. The renewed addition of selected bacterial strains guaranteed a sufficient number of these species within the biofilms.

After an additional incubation for 24 h, the medium was removed carefully and 20 µl of the tested substances were added to the biofilm. After 1 min, 180 µl of Wilkins Chalgren broth was added and the plates were incubated for 18h. Finally, after short washing the biofilm was carefully scraped, mixed by pipetting and cfu were enumerated after serial dilutions, spreading of each 25 µl on agar plates and incubation for 7 d.

Half of the 96-well plate was used for staining of the biofilm with the Kwasny and Opperman method [19]. After a manual plate washing, the biofilm was fixed by incubating the washed plate at 60ºC for 60 min. Finally the staining was performed with 0.06% (w/v) solution of crystal violet dissolved in dH_2O. 50 µl of the above stain was used per well and left for 5 minutes. After the staining procedure, the excess was removed by repeated washing. The amount of crystal violet bounded in each well was directly measured spectrophotometrically by measuring OD_{600} using microplate reader.

These experiments were made in two independent experiments in independent sextuplicates.

In addition, 4-d old biofilm samples on glass slides, treated with the test substances and processed as described above were stained with 0.1% acridine orange solution (Merck, Darmstadt, Germany) as a general nucleic acid stain. Samples were examined by using fluorescent microscope (Olympus BX51, Tokyo, Japan).

Furthermore, scanning electron microscope photographs were taken to visualize the results. Exemplarily each test substance was chosen. Samples were fixed in 2% glutaraldehyde in

cacodylate buffer for 30 min, washed twice with cacodylate buffer and dehydrated using a graded ethanol series (15 min each concentration). Following critical point drying, samples were sputter-coated with gold and examined with a ZEISS LEO-1530 Gemini (Carl Zeiss NTS GmbH) equipped with a field emission electron gun at 8 keV.

2.6. Statistical analysis

All data are presented as mean and standard deviation (SD). Data were compared using a one-way analysis of variance (ANOVA) with post-hoc comparisons of groups using LSD corrections. A *p*-value of 0.05 was considered to be statistically significant. However, in case of log10 cfu values the cfu log10 reductions are of importance, following only log10 cfu values are presented. SPSS software (version 22.0) was used for statistical analysis.

3. Results

All results were related in % to the working (commercially available) concentration (NaOCl gel and 0.1% CHX respectively).

3.1. Minimal inhibitory concentrations and minimal bactericidal concentrations

Except for one strain (*Parvimonas micra* ATCC 33270 in case of NaOCl gel) the MICs both of the CHX solution and NaOCl gel did not exceed 10% of the formulations' concentration. In general MICs of CHX were lower than those of the NaOCl gel. However, when differentiating between Gram-positives and Gram-negatives the difference was mainly due to the Gram-positive bacteria. NaOCl gel acted more growth inhibitory on Gram-negatives than on Gram-positives. Mainly NaOCl was the active compound of NaOCl gel. But the activating vehicle exerted also some activity on Gram-negatives; here a synergistic effect can be stated when comparing NaOCl gel with its compounds (Figure 1).

The MBC values were in general equal or one step higher than the corresponding MIC values. The difference was more or equal 2 steps for CHX against two microorganisms (incl. the

mixture) and for NaOCl gel against seven microorganisms (incl. the mixture). MBC values and individual MIC data are presented in Suppl. table 1.

3.2. Killing curves

Killing curves show the fast and total killing activity of 20% of the CHX solution and of the NaOCl gel. Only a few cells of *T. forsythia* were able to survive the exposure to the compounds. NaOCl gel was also tested in the lower concentrations of 5% and 10%. In part, a concentration dependent activity was visible (Figure 2).

3.3. Influence on formation of biofilm

A clear inhibition of biofilm formation by CHX is shown. Up to 6 h no bacteria were cultivable; after 24 and 48 h, less than 0.5 log10 cfu in mean were counted. The effect of NaOCl gel and its compounds was limited. The reduction was equally about 1.4 log10 cfu in mean both for the NaOCl gel as for NaOCl and the activating vehicle. After 48 h, the log10 cfu were the lower when the surface was coated with the activating vehicle (reduction about 2.7 log10 cfu in mean) than with NaOCl gel (reduction about 0.6 log10 cfu in mean) (Fig. 3).

3.4. Influence on 4 d old biofilm

In these assays, 100% of the formulations could act on the biofilms for 1 min before there was a dilution to 10%.

The number of cultivable bacteria was reduced by about 6 log10 cfu after application of NaOCl gel, being 1 log10 cfu more than after CHX. When NaOCl only was applied, no cultivable bacteria were detected. The activating vehicle itself did not have any effect (Fig 4A). These results did not correspond with those of the staining by cristal violet. Here, the highest values were measured for NaOCl ($p<0.01$ in comparison with control) followed by the untreated control and CHX. Lower staining was observed for the activating vehicle and the NaOCl gel ($p<0.05$, $p<0.01$ each in comparison with control) (Fig. 4B).

Staining with acridine orange showed a diffuse staining of the biofilm control, after application of CHX and the activating vehicle. The staining is less diffuse after NaOCl gel, whereas after NaOCl, only clear defined structures (bacteria) are stained (Fig. 5).

In all SEM photographs, many bacteria were visible. In part, damaged bacterial surfaces were detected after application of NaOCl and NaOCl gel. The matrix seemed to be less after NaOCl in comparison with the activating vehicle (Fig. 6).

4. Discussion

In this in vitro-study a sodium-hypochlorite gel was compared with a CHX solution which is still the gold standard in periodontal therapy. Growth inhibition and killing as well as the activity on a 6-species biofilm were evaluated.

MIC and MBC were determined by using standard procedures. The obtained values related to the available formulations revealed extremely low MICs of CHX and confirms findings from other studies [20, 21]. However, it should be mentioned that in the present study CHX was tested without any additives. It is well known that additives may influence disadvantageously the antimicrobial activity of commercial CHX formulations [22, 23]. The activity of NaOCl gel differed between Gram-positive and Gram-negative bacteria, growth of Gram-negatives is inhibited by lower concentrations. Interestingly, in the present study also the activating vehicle itself exerted certain antimicrobial activity. This selective inhibition may favor a more Gram-positive microbiota with *Actinomyces* spp., oral streptococci being in general more associated with periodontal health [24]. Moreover, it should be noted, that *Actinomyces* spp. are able to reduce nitrate to nitrite [25]. Killing of those bacteria by broad-spectrum antiseptics disturbs the physiological role of these oral bacteria in blood pressure control [26].

One important question to be answered was whether the application of NaOCl gel on a surface after mechanical plaque removal may prevent biofilm formation. This property is well known for chlorhexidine [27, 28] which is in part linked to its high substantivity [29]. Our in vitro study confirms the inhibition of biofilm formation for CHX, but for NaOCl gel no clear activity was seen. A slight inhibition (>1 log10 cfu) lasted only up to 24 h. However, NaOCl gel and in particular its component NaOCl clearly reduced vitality of a 4-d old biofilm. The used mode

simulated in vivo situation with an initial high (100%) concentration of the compounds followed by a dilution.

The overwhelming activity of NaOCl on the 4-d old biofilm in relation to the action against planktonic bacteria suggests an interference with the biofilm matrix. Staining of the biofilm mass confirmed reduced values after NaOCl gel. Crystal violet binds to negatively charged cell surface molecules and extracellular polysaccharides as a component of the biofilm matrix [30, 31]. After NaOCl the values were higher, about this unexpected finding can only be speculated. One explanation might be the exposure of hidden cell molecules to the surface or it might be a non-specific effect caused by the alkaline NaOCl.

Biofilm matrix consists of different components. Carbohydrates and proteins provide three-dimensional architectural integrity [32], enzymatic cleavage of matrix components is discussed to effect biofilm dispersal. Moreover, extracellular DNA as an important component of biofilm matrices [33] is essential in stress relaxation of biofilms [34]. Its degradation represents also an interesting approach for biofilm control [35]. NaOCl removes proteins; this property is well described when applied to dentin in caries [36] or endodontic lesions [37]. Besides of proteins NaOCl may target extracellular DNA in biofilm matrix. It is very efficiently used in laboratories to decontaminate DNA [38]. In this study, staining with acridine orange was made. Acridine orange stains single-stranded and double-stranded DNA [39]. DNA staining of our biofilms may underline that extracellular DNA but not the intracellular bacterial DNA is destroyed by NaOCl [39]. Recently by using eDNA extraction it was shown that enzymatic treatment with DNase I was not able to affect biofilm matrix in a two-species biofilm model [40]. The results indicate NaOCl gel as an interesting approach in combating biofilm-associated diseases; further research should analyze its interference with biofilm matrix in more detail.

Both fluorescent staining as well as SEM photographs show bacteria also in the NaOCl treated biofilms. It can be suggested that bacteria are not viable anymore. In particular, a clear damage was also visible on coccoid species which might be of interest as NaOCl had high MIC values against both species included in the biofilm assays. On the other hand, the exposure of biofilms to antimicrobials may lead to formation of dormant persisters [41]. The presence of persisters cannot be excluded, since the time after exposure to high concentrations of antimicrobials might be too short to stimulate multiplication of persisters visible as cfu on agar plates.

Since several years, sodium hypochlorite gel has been proven to be effective in removal of dentin caries and despite the fact that treatment time was longer, the patients preferred this

chemomechanical treatment to killing [42]. Furthermore, the data about its application in periodontal treatment are extremely rare and long-term studies are still missing. A study including 12 participants did not find any benefit in terms of decrease of instrumentation time, number of strokes to provide a calculus-free surface or subgingival calculus removal when sodium hypochlorite gel was applied adjunctive to SRP [43]. Similarly, the use of sodium hypochlorite gel as an adjunct to SRP presented no advantage for smear layer removal over scaling alone [44].

In summary, the present in-vitro study has shown that the new NaOCl gel acts antimicrobial in particular against Gram-negative species associated with periodontitis. Despite the fact that the NaOCl gel has failed to eliminate a multi-species biofilm, the vitality was clearly reduced and the matrix altered pointing to its high potential as an additive in mechanical therapy of periodontal disease.

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Conflict of Interest: The authors declare that they have no conflict of interest.

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Figure legends

Figure 1

MIC values of NaOCl gel incl. its components and chlorhexidine digluconate (related to the used formulations) against all species, Gram-positives and Gram-negatives

Figure 2

Killing of *Porphyromonas gingivalis* ATCC 33277, *Tannerella forsythia* ATCC 43037, *Parvimonas micra* ATCC 33270 and a 6-species mixture by different concentrations of NaOCl gel and 0.02% chlorhexidine digluconate (equivalent to 20% of a 0.1% formulation)

Figure 3

Influence of NaOCl gel incl. its components and 0.1% chlorhexidine digluconate on formation of a 6-species biofilm.

Surfaces were coated with test substances for 1 h before bacterial suspensions were added.

Figure 4

Influence of NaOCl gel incl. its components and 0.1% chlorhexidine digluconate on 4 days old 6-species biofilm.

NaOCl gel incl. its components and 0.1% chlorhexidine digluconate were added for 1 h to the biofilm, thereafter they were diluted 1: 9 for 18 h, before cfu (A) were determined and matrix was stained by cristal violet (B)

Figure 5

Staining of DNA in a 4 d old biofilm without (A) and after addition of 0.1% chlorhexidine digluconate (B), NaOCl gel (C) and its components NaOCl (D) and activating vehicle (E) for 1 h and after dilution 1: 9 for 18 h

Figure 6

Scanning electron microscopy photographs of a 4 d old biofilm without (A) and after addition of 0.1% chlorhexidine digluconate (B), NaOCl gel (C, D) and its components NaOCl (E) and activating vehicle (F) for 1 h and after dilution 1: 9 for 18 h

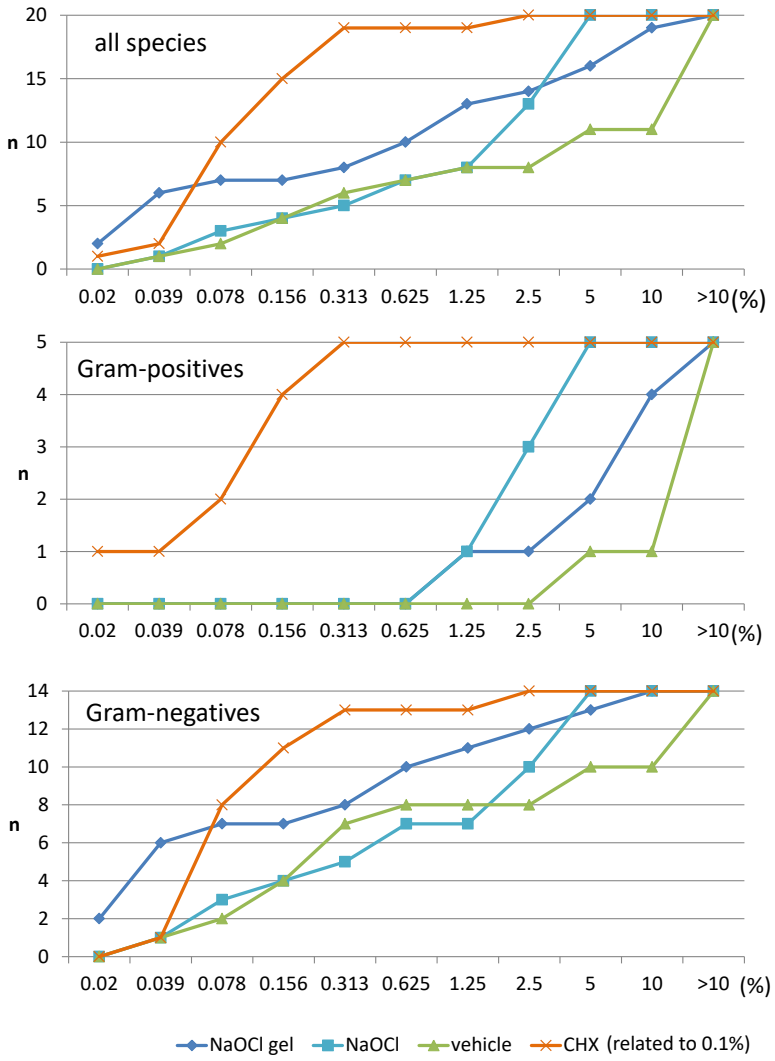
all species
n
0
5
10
15
20
0.02 0.039 0.078 0.156 0.313 0.625 1.25 2.5 5 10 >10 (%)
Gram-positives
n
0
1
2
3
4
5
0.02 0.039 0.078 0.156 0.313 0.625 1.25 2.5 5 10 >10 (%)
Gram-negatives
n
0
2
4
6
8
10
12
14
0.02 0.039 0.078 0.156 0.313 0.625 1.25 2.5 5 10 >10 (%)
NaOCl gel
NaOCl
vehicle
CHX (related to 0.1%)

Figure 1

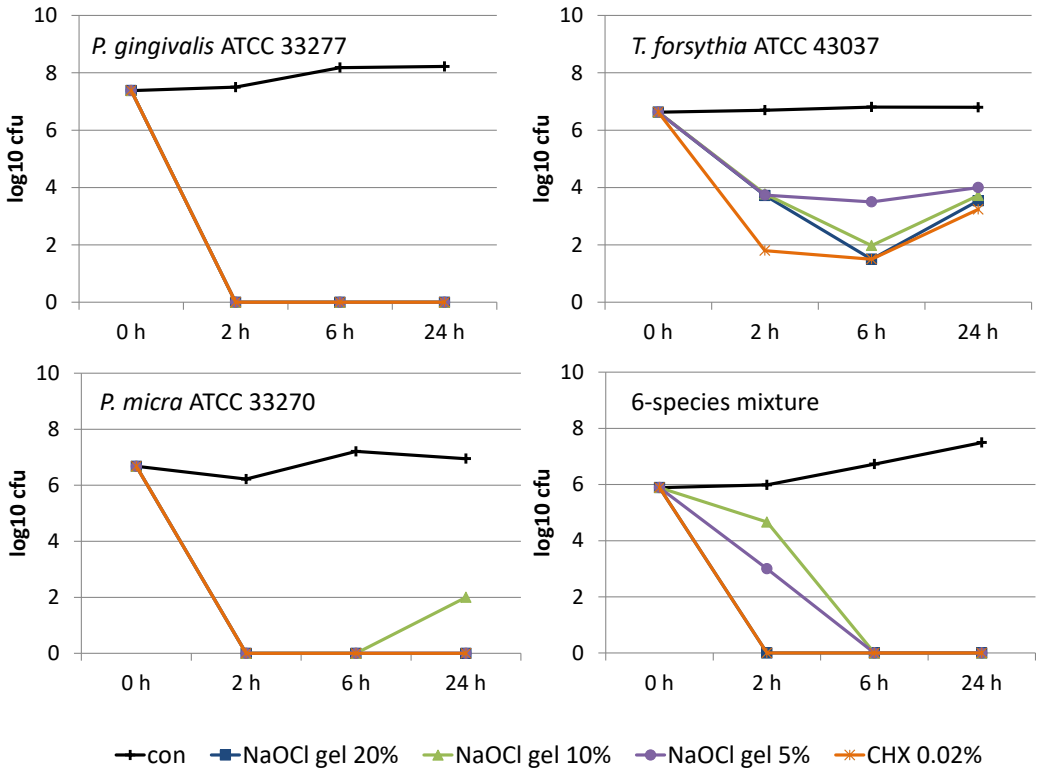
P. gingivalis ATCC 33277
T. forsythia ATCC 43037
P. micra ATCC 33270
6-species mixture
log10 cfu
0 h
2 h
6 h
24 h
con
NaOCl gel 20%
NaOCl gel 10%
NaOCl gel 5%
CHX 0.02%

Figure 2

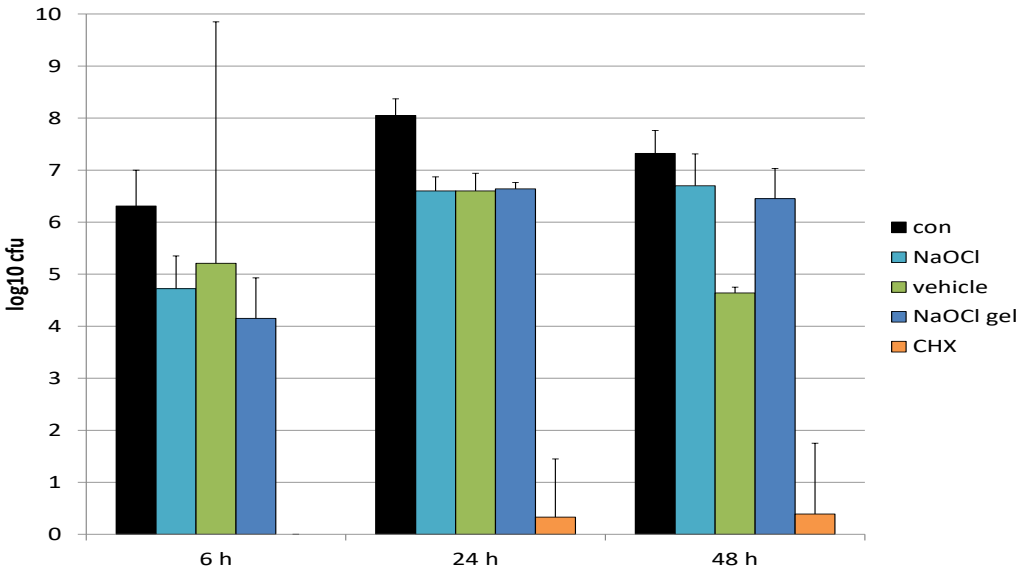
10
9
8
7
6
5
4
3
2
1
0
log10 cfu
6 h
24 h
48 h
con
NaOCl
vehicle
NaOCl gel
CHX

Figure 3

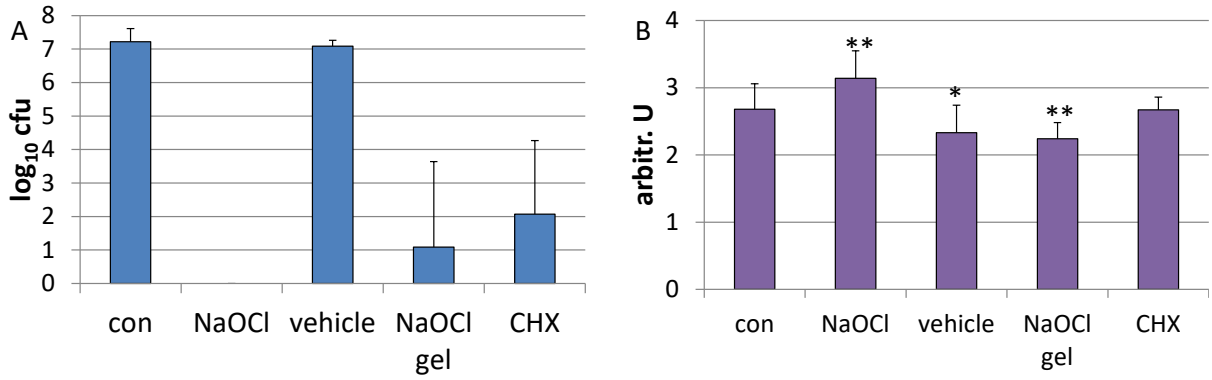

Figure 4

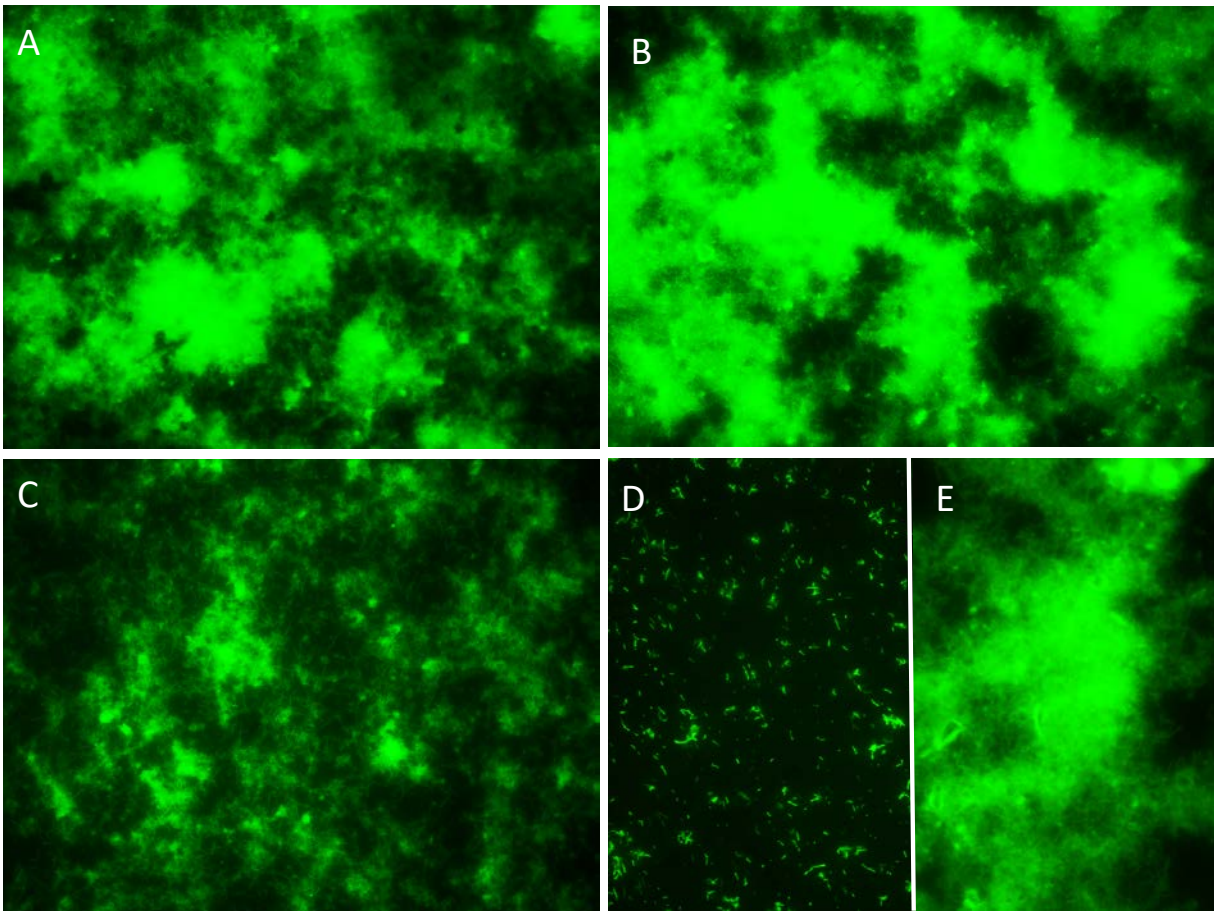

Figure 5

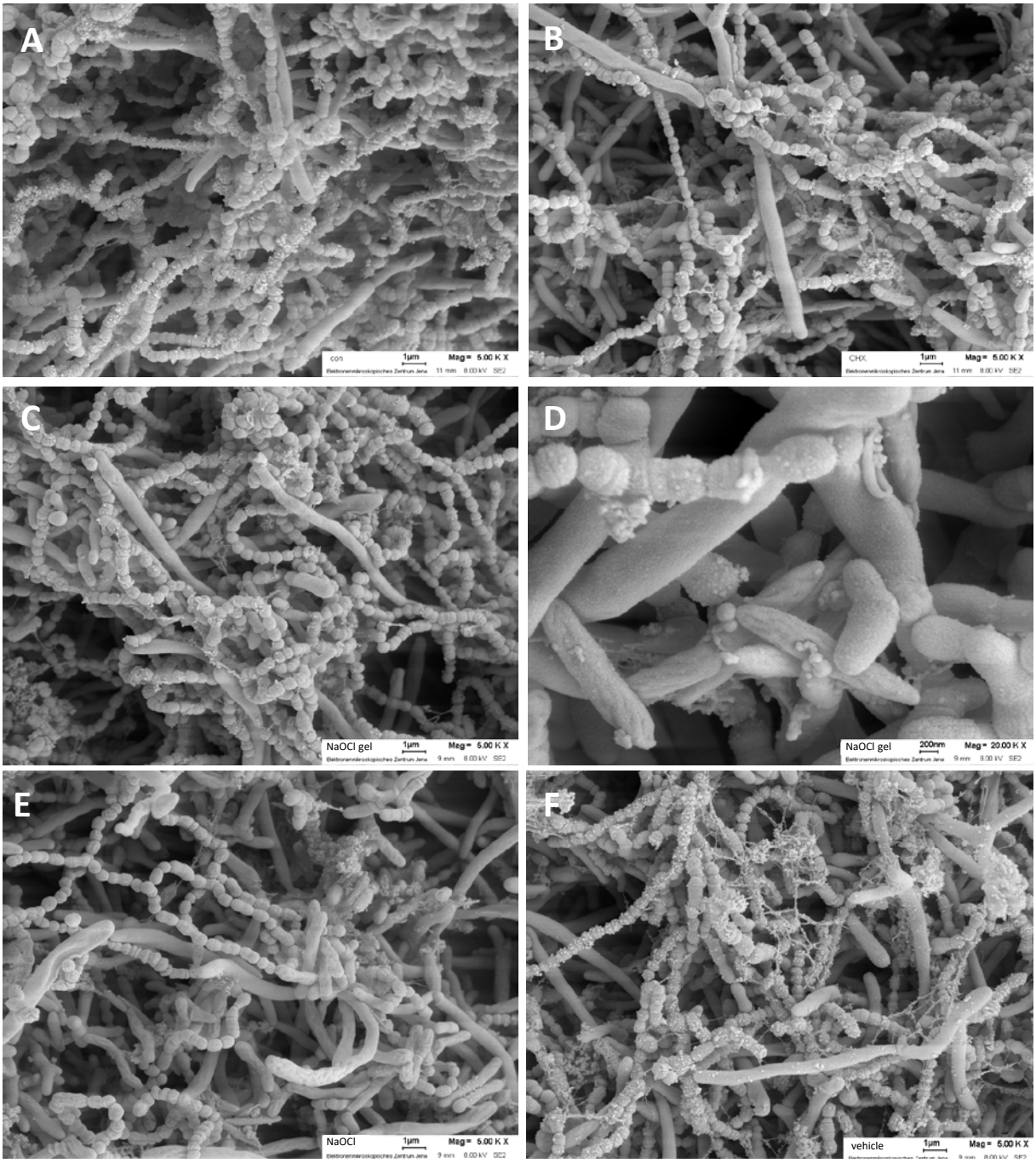
A
con
1µm
Mag= 5.00 K X
B
CHX
1µm
Mag= 5.00 K X
C
NaOCl gel
1µm
Mag= 5.00 K X
D
NaOCl gel
200nm
Mag= 20.00 K X
E
NaOCl
1µm
Mag= 5.00 K X
F
vehicle
1µm
Mag= 5.00 K X

Figure 6

Suppl. Table 1

MIC and MBC values of NaOCl gel and chlorhexidine digluconate against studied bacterial strains and the 6-species mixtures (values are given in % of the available formulation (NaOCl gel and 0.1% chlorhexidine digluconate)

	MIC of NaOCl gel and its components			**MIC of chlorhexidine digluconate**	**MBC of NaOCl gel and its components**			**MBC of chlorhexidine digluconate**
	NaOCl gel	**NaOCl**	**Vehicle**		**NaOCl gel**	**NaOCl**	**Activating vehicle**	
Porphyromonas gingivalis ATCC 33277	0.625	2.500	0.156	0.156	0.625	2.500	0.625	0.313
P. gingivalis J430	0.039	0.039	0.078	0.078	0.078	0.078	0.156	0.078
P. gingivalis ORA	0.039	0.313	0.312	0.078	0.156	0.625	2.500	0.156
P. gingivalis M5-1-2	0.039	0.625	0.156	0.078	0.078	1.250	0.313	0.156
Tannerella forsythia ATCC 43037	0.020	0.156	0.312	0.078	0.039	0.156	0.312	0.156
T. forsythia B13216	0.020	0.078	0.039	0.078	0.078	0.156	0.156	0.078
T. forsythia RA12	0.039	0.039	0.156	0.039	0.078	0.078	0.156	0.039
T. forsythia O20	0.078	0.313	0.313	0.078	0.156	0.625	0.313	0.078
Fusobacterium nucleatum ATCC 25586	1.250	5.000	>10.000	0.156	5.000	5.000	>10.000	0.156
Streptococcus gordonii ATCC 10558	10.000	5.000	>10.000	0.078	10.000	2.500	>10.000	0.156
Actinomyces naeslundii ATCC 12104	10.000	1.250	>10.000	0.156	10.000	2.500	>10.000	0.156
Parvimonas micra ATCC 33270	>10.000	2.500	>10.000	0.313	>10.000	2.500	>10.000	0.625
Prevotella intermedia ATCC 25611	0.313	5.000	5.000	0.156	0.313	5.000	10.000	0.313

Aggregatibacter actinomycetemcomitans ATCC 33384	10.000	5.000	>10.000	0.313	10.000	10.000	>10.000	0.625
Campylobacter rectus ATCC 33238	0.625	2.500	>10.000	0.078	5.000	1.250	>10.000	0.078
Eikenella corrodens ATCC 23834	2.500	5.000	>10.000	2.500	5.000	5.000	>10.000	5.000
Filifactor alocis ATCC 33099	1.250	2.500	>10.000	0.156	5.000	5.000	>10.000	0.156
Capnocytophaga gingivalis ATCC 33624	5.000	2.500	5.000	0.313	5.000	5.000	>10.000	0.625
Eubacterium nodatum ATCC 33270	5.000	5.000	5.000	0.020	>10.000	5.000	>10.000	0.156
6-species mixture	1.250	5.000	>10.000	0.313	10.000	5.000	>10.000	1.250