Activity of chlorhexidine formulations on oral microorganisms and periodontal ligament fibroblasts

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Abstract

Given the importance of microorganisms in the pathogenesis of the two most prevalent oral diseases (i.e. caries and periodontitis), antiseptics are widely used. Among the antiseptics chlorhexidine (CHX) is still considered as gold standard. The purpose of this in-vitro-study was to determine the antimicrobial activity of new CHX digluconate containing formulations produced in Switzerland. Two test formulations, with 0.1% or 0.2% CHX (TestCHX0.1, TestCHX0.2) were compared with 0.1% and 0.2% CHX digluconate solutions (CHXph0.1, CHXph0.2) without additives and with a commercially available formulation containing 0.2% CHX digluconate (CHXcom0.2). The minimal inhibitory concentrations (MIC) of the CHX formulations were determined against bacteria associated with caries or periodontal disease. Then the anti-biofilm activities of CHX preparations were tested regarding inhibition of biofilm formation or against an existing biofilm. Further, the cytotoxicity of the CHX preparations against periodontal ligament (PDL) fibroblasts was measured. There were no or only minor differences of the MIC values between the CHX preparations. Except for 0.1% CHXph, all formulations blocked the formation of the biofilms. When analyzing the effect on an established “cariogenic” biofilm, the TestCHX0.2 was most active (- 3.5 log₁₀ colony forming units (cfu); p<0.001 vs. control). The cfu counts of an established periodontal biofilm were most decreased by CHXcom0.2 (-3.9 log₁₀), followed by TestCHX0.2 (-3.34 log₁₀) and CHXph0.2 (-3.0 log₁₀ cfu) (each p<0.001 vs. control). All CHX preparations reduced the viability of the PDL fibroblasts. Taken together, the Swiss made test mouth rinses have an equal or better antibacterial and anti-biofilm activity as the respective CHX solutions and can be recommended.
Introduction

Caries and periodontitis are the most common chronic diseases in mankind with a very high prevalence. According to recent published data, untreated caries in permanent teeth was the most prevalent health condition affecting 35% of the people world-wide and periodontitis was the sixth most prevalent one affecting about 10% of the population in 2010 (PERES ET AL. 2019). Dental caries is characterized by mineral loss being the result of a shift in the microbiota in the presence of sugar and towards a microbiota dominated by acidogenic and aciduric bacteria (including mutans streptococci) (NYVAD ET AL. 2013).

The pathogenesis of periodontal disease is considered to be the result of a disturbed homeostasis within tooth-supporting tissues by dysbiotic microbiota leading to inflammation and slowly eroding periodontal tissues (DARVEAU ET AL. 2012). Bacterial species associated with periodontal health are among others Streptococcus gordonii, Actinomyces naeslundii, Capnocytophaga gingivalis, Eikenella corrodens, those more present in gingival inflammation Parvimonas micra, Prevotella intermedia and several Tannerella ssp., and in case of periodontal destruction joined by Filifactor alocis, Porphyromonas gingivalis, Treponema denticola and Campylobacter rectus, thereby Fusobacterium nucleatum represents a core species (DIAZ ET AL. 2016).

Given the importance of microorganisms in the pathogenesis of the major oral diseases, antiseptics are widely used. Among the antiseptics chlorhexidine (CHX) is still the gold standard although side effects, e.g. tooth surface discoloration occur (SLOT ET AL. 2014). As recently reviewed, the beneficial effects of CHX are confirmed in reducing of plaque accumulation, in preventing tooth caries, gingivitis, and periodontitis (KARPINSKI&SZKARADKIEWICZ 2015). Clinically, the application of CHX mouth rinse in addition to tooth brushing is more effective in reducing plaque and gingival inflammation than tooth brushing alone (ARORA ET AL. 2014). However, antibacterial activity of CHX formulations can be different from aqueous solutions of the compound (EICK ET AL. 2011).
The purpose of this in-vitro-study was to determine the antimicrobial activity of new CHX digluconate containing formulations produced in Switzerland and to compare them with chlorhexidine digluconate without additives as well as with a commonly used commercially available product.

Materials and Methods

CHX formulation

Four CHX solutions were used. Two test formulations, one contained 0.1% (TestCHX0.1), the other 0.2% (TestCHX0.2) CHX digluconate (CHX MOUTH WASH CHX 0.1%, CHX MOUTH WASH CHX 0.2%, Top Caredent AG, Zurich, Switzerland), were included in the assays. The activities of the formulations were compared with 0.1% and 0.2% CHX (CHXph) digluconate solutions diluted with Aqua dest. from a 2% solution prepared at the pharmacy of the Insel University Hospital Bern. Further a commercially available formulation with 0.2% CHX digluconate (CHXcom0.2); (Chlorhexamed forte 0.2%; GlaxoSmithKline Consumer Healthcare, Brentford, UK) was used. The given CHX concentrations and the other ingredients (based on the manufacturers’ information each) are presented in Table 1.

Bacterial strains

The following bacterial species were used in the assays:

1. *Streptococcus gordonii* ATCC 10558
2. *Actinomyces naeslundii* ATCC 12104
3. *S. mutans* ATCC 25175
4. *S. sobrinus* ATCC 33478
5. *Lactobacillus acidophilus* ATCC 11975
6. *Fusobacterium nucleatum* ATCC 25586
7. *Parvimonas micra* ATCC 33270
8. *Porphyromonas gingivalis* ATCC 33277
9. **Tannerella forsythia ATCC 43037**

Before starting the experiments, strains were subcultivated on Tryptic-soy agar plates (Oxoid, Basingstoke, GB) with 5% sheep blood (and with 10 mg/l N-acetylic muramic acid (Merck KGaA, Darmstadt, Germany).

The minimal inhibitory concentrations of the CHX formulations were determined against all the bacteria listed above. The biofilms always consisted of *S. gordonii* ATCC 10558, and *Actinomyces naeslundii* ATCC 12104, added by *S. mutans* ATCC 25175, *S. sobrinus* ATCC 33478 and *L. acidophilus* ATCC 11975 in case of the cariogenic biofilm and by *Fusobacterium nucleatum* ATCC 25586, *Parvimonas micra* ATCC 33270, *Porphyromonas gingivalis* ATCC 33277 and *Tannerella forsythia* ATCC 43037 in case of the periodontal biofilm.

**Determination of minimal inhibitory concentrations (MIC)**

The minimal inhibitory concentrations (MICs) of the CHX formulations/solutions were determined by using the micro-broth dilution technique. First a two-fold dilution series from the CHX formulations/solutions was prepared. The highest concentration was equivalent to 0.004% CHX (final concentration 0.002%). Then each 100 µl of the respective dilution was pipetted in a well of a 96-well-microtiter plate. The bacterial strain to be tested was suspended in 0.9 w/v NaCl and thereafter added in a ratio 1 : 50 to two-fold concentrated Wilkins-Chalgren broth and then also each 100 µl were pipetted per well. After an incubation time of 42 h (18 h aerobic conditions) with 10% CO₂ or anaerobically at 37°C, the growth of microbes was monitored visually and by subcultivation. The MIC was defined as the lowest concentration without visible turbidity (or with clear growth inhibition).

The experiments were made in independent replicates.

**Biofilm experiments**
The bacterial strains were suspended in 0.9% w/v NaCl according to McFarland 4. Then the suspensions were mixed by adding 1 part S. gordonii ATCC 10558, 2 parts A. naeslundii ATCC 12104 and each 4 parts of the other bacteria. This suspension was finally mixed with the nutrient broth (Wilkins-Chalgren broth) in a ratio 1 : 9.

Two different scenarios were simulated, a) the application of a mouth rinse after a mechanical removal of biofilm (influence on formation of biofilms) and b) if there is any effect on a biofilm, which has been already established (established biofilm).

a) Influence on biofilm formation: The wells of a 96-well plate were covered with 25 µl of a 10% solution of the test substances and had been left in a safety cabinet for 60 min. Then, 25 µl of a protein solution (1.5% bovine serum albumin in phosphate buffered saline (PBS)) had been added for 30 min. Finally, 200 µl of the bacterial suspension / nutrient broth mixture were pipetted to each well. The final concentration of the CHX formulations/solutions in the assay was 1% of the respective formulation/solution (meaning either 0.001% or 0.002% CHX). The incubation occurred at 37°C with 10% CO₂ (cariogenic biofilm) or under anaerobic conditions (periodontal biofilm). At 6 h, the nutrient broth was removed and after a short and careful washing, 250 µl 0.9% w/v NaCl were pipetted per well. The biofilms were removed from the surface by scraping, mixing, and by ultrasonication. After mixing by pipetting, a serial dilution was made and the total colony forming unit (cfu) counts were assessed.

Established biofilm: The biofilms were formed as described before. The wells were coated first with 25 µl of the protein solution, before the bacterial suspension / nutrient broth mixture was added. The cariogenic biofilm had been incubated for 48 h and the periodontal biofilm for 3.5 d. In case of the periodontal biofilm, P. gingivalis and T. forsythia were added again after 48 h. At 48 h (cariogenic biofilm) or 3.5 d (periodontal biofilm) the nutrient broth was removed and after short and careful washing, 25 µl of the test substances were applied for 1 min. Then, nutrient broth (225 µl) was added and the biofilms were analyzed after 1 h of incubation. As before, the total cfu counts were assessed. In addition, the quantification of the biofilms (“biofilm mass”) was made after staining with crystal violet according to recently published
protocols (KWASNY&OPPERMAN 2010). In short, after rinsing and fixing the biofilms at 60°C for 60 min, 50 µl of a 0.06% (w/v) crystal violet (Sigma-Aldrich Chemie GmbH) solution were pipetted per well and left in place for 10 min. Finally the staining was assessed by using a plate reader (ELx808, Biotek Instruments, Winooski, VT, USA) at 600 nm. The metabolic activity of the biofilm suspension was determined using Alamar blue; as a measure of cell viability it quantifies the chemical reduction of Alamar blue (resazurin) (PETTIT ET AL. 2005). Alamar blue (alamarBlue® reagent, Thermo Fisher Scientific Inc., Waltham, MA, USA) was mixed with the nutrient media in a ratio 1 : 20 and each 100 µl were pipetted per well to the biofilm. The microtiter-plate biofilm had been incubated for 1 h at 37°C, before the absorbances were measured at 570 nm against 600 nm by using the microplate reader (ELx808, Biotek).

3. Determination of a potential cytotoxicity

Periodontal ligament (PDL) fibroblasts were harvested from extracted donor teeth from patients who had been informed about the use of their cells in research and given written consent. According to the guidelines, no previous approval from the Cantonal ethical committee KEK was necessary as the biomaterials were categorized as “irreversibly anonymised”. The same method was used as described before (EICK ET AL. 2011). The PDL fibroblasts were grown to confluent monolayers in 96-well-plates. After aspirating the cell culture media and after a two-fold washing with PBS, the test substances (control Aqua dest.) were added. After 1 min of exposure, the test solutions were removed and replaced by the cell cultivation media for 1 h. Finally, the MTT tetrazolium colorimetric assay (MOSMANN 1983) was used to determine the capacity of mitochondrial enzymes as a measure of cell viability. In the experiments, cells obtained from two different donors were included.

The biofilm and cytotoxicity experiments were made in two independent experiments in each independent quadruplicates. The software SPSS 25.0 (IBM SPSS Statistics, Chicago, IL, USA)
was used for statistical analysis. ANOVA followed by Bonferroni compared the different results. The level of statistical significance was set to $p = 0.05$.

**Results**

*Minimal inhibitory concentration (MIC) values*

There were no or only minor differences of the MIC values regarding the CHX concentrations between the CHX formulations. Related to the CHXph, the difference never exceeded one dilution step (Table 2).

*“Cariogenic” biofilm*

Except for CHXph0.1 all formulations blocked biofilm formation. The CHXph0.1 formulation decreased the counts in biofilm by about $5.7 \log_{10}$ cfu. This indicates, that all differences vs. control were statistically significant (each $p<0.001$), further when comparing formulations with the respective concentration of CHX in the solution from the pharmacy, there was a better performance of the TestCHX0.1 vs. CHXph0.1 solution ($p=0.001$) (Figure 1).

Analyzing the effect on an established biofilm, the TestCHX0.2 was most active, the cfu counts decreased in mean by $3.5 \log_{10}$ vs. control ($p<0.001$). Further, there were statistically significant differences between CHXcom0.2 (-2.7 log10 cfu; $p=0.003$) and TestCHX0.1 (-2.4 log10; $p=0.012$) vs. control. In comparison with the CHX solutions from pharmacy, the TestCHX0.2 was more active than CHXph0.2 ($p=0.005$) (Figure 2A). There were no statistically significant differences in the biofilm mass (Figure 2B). In all the biofilms treated with CHX solutions, the metabolic activity was highly reduced vs. control (each $p<0.001$). There were also statistically significant differences between the Test CHX formulations vs. CHX solutions from pharmacy; the TestCHX0.1 reduced the activity more than CHXph0.1 ($p<0.001$) (Figure 2C).
“Periodontal” biofilm

Except for CHXph0.1, all the formulations completely blocked biofilm formation. The CHXph0.1 formulation decreased the counts in biofilm by about 7 log_{10} cfu (differences vs. control each $p<0.001$) (Figure 3).

Analyzing the effect on an established biofilm, all the CHX formulations decreased statistically significantly the cfu counts. CHXcom0.2 (-3.9 log10) was most active, followed by TestCHX0.2 (-3.34 log10) and CHXph0.2 (-3.0 log10 cfu) (each $p<0.001$ vs. control; Figure 4A). There were no statistically significant differences in the biofilm mass (Figure 4B). Similarly, also regarding the metabolic activity, the differences were minor. Only CHXcom0.2 reduced statistically significantly the metabolic activity vs. the untreated control ($p=0.023$) (Figure 4C).

Viability of periodontal ligament fibroblasts after exposure to CHX formulations

All the CHX formulations reduced statistically significantly the viability of the PDL fibroblasts vs. control. There were no statistically significant differences between the CHX preparations (Figure 5).

Discussion

In the present study, new CHX digluconate containing formulations developed and made in Switzerland, were compared with CHX digluconate solutions and a long-term available commercial product. The results show an antibacterial and antibiofilm activity comparable to the solutions from pharmacy and to an available commercial product. However, the CHX digluconate containing formulation showed similar cytotoxicity as other CHX preparations to periodontal fibroblasts.
An in-vitro study was performed in order to compare the different groups in a standardized manner. However, when interpreting the results, it is always important to keep in mind that such a model can impossibly reflect the in-vivo situation. Nevertheless, we tried to simulate both a potential activity on the biofilm formation and the activity on an already established biofilm. In case of the biofilm formation, first the surface was coated with a 10% solution of the CHX preparation and thereafter, a protein solution was added. Finally, the biofilm was cultured in a 1% solution. In the second attempt, a biofilm was first cultured before adding the CHX preparations. Subsequently, the undiluted preparation was added for 1 min before diluting to 10%. However, the fact that this was not a continuous model might represent a further limitation of the present study.

The MIC values did not differ between the formulations and the solutions. They underline the high antimicrobial activity of CHX both to bacteria associated with caries and periodontal disease. The very low MIC values against S. mutans are in the range of recent results on 10 clinical isolates (SO YEON & SI YOUNG 2019). Bacteria associated with periodontal disease were also very susceptible, the present study confirmed the results on MIC values published before (MCBAIN ET AL. 2003, MENDES ET AL. 2020)

It is well known that the antibacterial activity of CHX formulations might be affected by additives. E.g., CHX formulations containing an anti-discoloration system reduced the side-effects of staining, but resulted also in less antibacterial activity as shown in in-vitro models (EICK ET AL. 2011). The in-vitro data are in agreement with findings from clinical studies which have shown that CHX with added anti-discoloration system was less efficient in reducing plaque scores (GRAZIANI ET AL. 2015). Thus, an important aspect of the present study is the fact that the test formulations in the present study were not less active than the control CHX solutions. Therefore, it can be concluded that the additives included in the formulations do not negatively interfere with the antimicrobial activity.

In contrast, regarding the “cariogenic” biofilm, the test formulations had a higher anti-biofilm activity. This might be linked with the benzalkonium chloride content of the formulations. Benzalkonium chloride is a biocide used in ophthalmology (HEDENGRAN ET AL. 2020) and in
wound dressings (JIN ET AL. 2015). In dentistry, it is discussed as an additive to orthodontic adhesives (OZEL ET AL. 2017), dentine bonding materials (FLURY ET AL. 2017) and dentures liners (ALTINCI ET AL. 2018). Benzalkonium chloride was shown to inhibit biofilm formation of a three species biofilm on dentine disks, the underlying mechanism was discussed as a less attachment to the surface (JARAMILLO ET AL. 2012). A comparable effect was also seen when using bacteria such as Staphylococcus aureus, Pseudomonas aeruginosa and Candida ssp. (STOFFEL ET AL. 2020). An antibacterial activity was found against S. mutans (OZEL ET AL. 2017, ALTINCI ET AL. 2018), however no data are available about an activity against periodontopathogens, e.g. F. nucleatum, P. gingivalis, T. forsythia.

Our results underline once more, that CHX might be preventive in retarding biofilm formation but it has only limited activity on already formed biofilms. This supports the well-known fact that the biofilm has to be first mechanically removed. This is in accordance with recommendations set in different fields in dentistry. In periodontal therapy, it is recommended to perform professional mechanical biofilm removal; mouth rinses have a beneficial effect in reducing intra-oral halitosis (SANZ ET AL. 2015). Prosthodontic patients should use a toothbrush and toothpaste and only shortly in sporadic cases CHX (CORTELLI ET AL. 2014). Similarly, in individuals with high risk for caries, the regimen includes a fluoride-containing toothpaste and the use of chlorhexidine-mouth rinse for short periods (NASCIMENTO 2013).

Meanwhile, the toxic activity of CHX against cells cultured in laboratory is known. E.g., in a recent study primary myoblasts, osteoblasts and fibroblasts were exposed to different concentrations of CHX; after 1 min of exposure to 0.02% CHX, survival of all cells dropped down to close zero (LIU ET AL. 2020). The results of the present study confirm a cytotoxicity also for the test formulations. Besides CHX, the test formulations contain benzalkonium chloride, which was also found to negatively affect the cell viability. Using a standardized cell line recommended for cytotoxicity testing after a 30 min exposure the IC\textsubscript{50} values were 87 mg/L for CHX and 64 µg/L for benzalkonium chloride (MULLER&KRAMER 2008). In a recent systematic review, 13 out of 51 included studies reported adverse effects of CHX mouthrinses on oral mucosa (JAMES ET AL. 2017). However, the same systematic provided significant
evidence for plaque reduction and inhibition of mild gingival inflammation following the use of CHX mouth rinses.

In summary, the Swiss made test mouth rinses have an equal or better antibacterial and anti-biofilm activity as the respective CHX solutions and can be recommended, however as other CHX preparations they are cytotoxic.

Acknowledgements

The authors acknowledge the excellent technical assistance by Prashantnj Sivapatham (Department of Periodontology, Laboratory of Oral Microbiology, School of Dental Medicine, University of Bern).

Zusammenfassung


Material und Methoden: Die zwei Testformulierungen mit 0,1% oder 0,2% CHX Diglukonat (TestCHX0.1, TestCHX0.2) wurden mit 0,1% und 0,2%igen CHX-Diglukonat-Lösungen (CHXph0.1, CHXph0.2) ohne Zusatzstoffe und mit einem breit angewendeten kommerziell erhältlichen Produkt mit 0,2% CHX-Diglukonat (CHXcom0.2) verglichen. Zunächst wurde die minimale Hemmkonzentration (MHK) der CHX-Formulierungen gegenüber neun oralen Bakterien (assoziiert mit Karies oder Parodontitis) ermittelt. Anschliessend wurde bestimmt, ob eine mögliche hemmende Wirkung gegenüber einer Biofilm-Neubildung besteht und ob ein existierender Biofilm beeinflusst werden kann. Hierfür wurde jeweils ein «kariogener» Biofilm,

Resultate: Die direkte antimikrobielle Aktivität der Testformulierungen (MHK) unterschied sich nicht von der der Vergleichsformulierungen. Die Bildung von kariogenem und parodontalem Biofilm wurde durch alle Formulierungen mit Ausnahme der 0,1%igen CHX-Lösung ohne Zusatzstoffe blockiert. In Bezug auf einen bereits bestehenden “kariogenen” Biofilm zeigte die TestCHX0.2-Formulierung die höchste keimreduzierende Wirkung (-3.5 log10 KBE) von allen geprüften CHX-Formulierungen. Bei einem bereits vorhandenen «parodontalen» Biofilm wies die CHXcom0.2-Formulierung (-3.9 log10 KBE) die höchste Aktivität auf, gefolgt von der TestCHX0.2-Formulierung (-3.34 log10 KBE) und der CHXph0.2-Lösung (-3.0 log10 cfu KBE). Die CHX-Zubereitungen reduzierten deutlich die Stoffwechselaktivität im «kariogenen» Biofilm, nicht aber im parodontalen «Biofilm». Bei beiden Biofilmen wurde deren Quantität durch die CHX-Formulierungen nicht beeinflusst. Neben der guten antimikrobiellen Wirksamkeit konnte aber auch bei allen CHX-Formulierungen ein zytotoxischer Effekt auf parodontale Fibroblasten nachgewiesen werden.

Diskussion: Die in der Schweiz hergestellten CHX-Formulierungen haben verglichen mit CHX-Lösungen ohne Zusatzstoffe eine gleiche oder teilweise bessere In-vitro-Wirkung auf orale Bakterien und Biofilme und können deshalb zur Anwendung empfohlen werden. Auffällig ist deren guten Wirksamkeit gegenüber dem «kariogenen» Biofilm, was im Zusammenhang mit dem Inhaltsstoff Benzalkoniumchlorid stehen könnte. Bei einem bereits vorhandenen Biofilm
is the Wirkung von CHX-Formulierungen eingeschränkt. Das unterstreicht die Notwendigkeit einer vorgängigen mechanischen Biofilmentfernung.

References


Table 1

Ingredients of the commercial chlorhexidine digluconate formulations (according to the manufacturers’ descriptions)

<table>
<thead>
<tr>
<th></th>
<th>TestCHX0.1</th>
<th>TestCHX0.2</th>
<th>CHXcom0.2</th>
<th>CHXph</th>
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<tr>
<td>CHX digluconate</td>
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</tr>
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<td>Other ingredients</td>
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<td>Aqua pure</td>
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<tr>
<td>Peppermint oil</td>
<td>Peppermint oil</td>
<td>Macrogolglyceroli hydroxystearas</td>
<td>Sorbitol</td>
<td>Vanillum et alia (mentha piperata)</td>
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<tr>
<td>Xylitol</td>
<td>Xylitol</td>
<td>Benzalkonium chloride</td>
<td>Benzalkonium chloride</td>
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<tr>
<td>Benzalkonium chloride</td>
<td></td>
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<td></td>
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</tbody>
</table>

Table 1

Minimal inhibitory concentrations of chlorhexidine (CHX) formulations

Results are given related to the % of CHX within the formulations

<table>
<thead>
<tr>
<th></th>
<th>TestCHX0.1</th>
<th>TestCHX0.2</th>
<th>CHXcom0.2</th>
<th>CHXph</th>
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<td>A. naeslundii ATCC 12104</td>
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</tr>
<tr>
<td>T. forsythia ATCC 43037</td>
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Colony forming units (cfu) counts (means and SD) in biofilms after covering surface with 10 % of chlorhexidine formulations (test formulation 0.2% (TestCHX0.2), test formulation 0.1% (TestCHX0.1), commercially CHX formulation 0.2% (CHXcom0.2) and 0.1% and 0.2% CHX solutions from pharmacy (CHXph0.2 and CHXph0.1) and subsequent formation of a “cariogenic” biofilm consisting of five species in 1% concentrations of the formulations for 6 h.
Colony forming units (cfu) counts (A), mass (B) and metabolic activity (C) of “cariogenic” biofilms after culturing for 48 h and subsequent exposing to chlorhexidine formulations (test formulation 0.2% (TestCHX0.2), test formulation 0.1% (TestCHX0.1), commercially CHX formulation 0.2% (CHXcom0.2) and 0.1% and 0.2% CHX solutions from pharmacy (CHXph0.2 and CHXph0.1) for 1 min and following incubation in a concentration of 10% of the formulations for 1 h

Presented are means and SD as well as statistically significant differences vs. control and CHX solutions from pharmacy in the respective concentration.
Figure 3

Colony forming units (cfu) counts (means and SD) in biofilms after covering surface with 10% of chlorhexidine formulations (test formulation 0.2% (TestCHX0.2), test formulation 0.1% (TestCHX0.1), commercially CHX formulation 0.2% (CHXcom0.2) and 0.1% and 0.2% CHX solutions from pharmacy (CHXph0.2 and CHXph0.1) and subsequent formation of a “periodontal” biofilm consisting of six species in 1% concentrations of the formulations for 6 h.

** p<0.01 vs. control
Colony forming units (cfu) counts (A), mass (B) and metabolic activity (C) of “periodontal” biofilms after culturing for 48 h and subsequent exposing to chlorhexidine formulations (test formulation 0.2% (TestCHX0.2), test formulation 0.1% (TestCHX0.1), commercially CHX formulation 0.2% (CHXcom0.2) and 0.1% and 0.2% CHX solutions from pharmacy (CHXph0.2 and CHXph0.1) for 1 min and following incubation in a concentration of 10% of the formulations for 1 h.

Presented are means and SD as well as statistically significant differences vs. control. **p<0.01; *p<0.05 vs. control.
Viability (mean ± SD) of periodontal ligament fibroblasts measured by the MTT assay after being exposed to chlorhexidine formulations (test formulation 0.2% (TestCHX0.2), test formulation 0.1% (TestCHX0.1), commercially CHX formulation 0.2% (CHXcom0.2) and 0.1% and 0.2% CHX solutions from pharmacy (CHXph0.2 and CHXph0.1) for 1 min and subsequent replacement by cell culture media for 1 h.

The viability related to the control (1 min Aqua dest.) is presented.