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# Activity of chlorhexidine formulations on oral microorganisms and periodontal ligament fibroblasts

#### KEYWORDS

Biofilm  
Antiseptics  
Cytotoxicity

#### SUMMARY

Given the importance of microorganisms in the pathogenesis of the two most prevalent oral diseases (i.e., caries and periodontitis), anti-septics 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 ( $-3.5 \log_{10}$  colony-forming units (cfu);  $p < 0.001$  vs. control). The cfu counts of an established periodontal biofilm were most decreased by CHXcom0.2 ( $-3.9 \log_{10}$ ), followed by TestCHX0.2 ( $-3.34 \log_{10}$ ) and CHXph0.2 ( $-3.0 \log_{10}$  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* and *Eikenella corrodens*, those more present in gingival inflammation *Parvimonas micra*, *Prevotella intermedia* and several *Tannerella* ssp., and in case of periodontal destruction they are 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 and 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) are presented in Table I.

### 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 add-

**Tab. I** Ingredients of the commercial chlorhexidine digluconate formulations (according to the manufacturers' descriptions)

	TestCHX0.1	TestCHX0.2	CHXcom0.2	CHXph
<b>CHX digluconate</b>	0.1%	0.2%	0.2%	2%
<b>Other ingredients</b>	Aqua pure	Aqua pure	Aqua pure	Aqua pure
	Peppermint oil	Peppermint oil	Macrogolglyceroli hydroxystearas	
	Xylitol	Xylitol	Sorbitolum	
	Benzalkonium chloride	Benzalkonium chloride	Vanillum et alia (mentha piperata)	

ed 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% CO<sub>2</sub> 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 4 parts each 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 biofilm formation) 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% CO<sub>2</sub> (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.

b) 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).

### 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 (Tab. II).

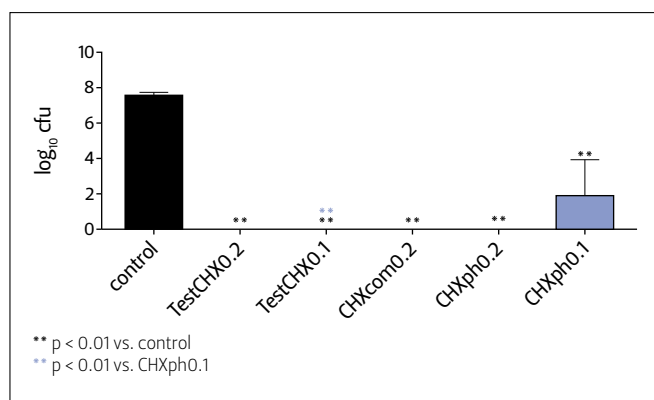
### Cariogenic biofilm

Except for CHXph0.1 all formulations blocked biofilm formation. The CHXph0.1 formulation decreased the counts in biofilm by about 5.7 log<sub>10</sub> cfu. This indicates that all differences vs. control were statistically significant ( $p < 0.001$ ), 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 ( $p = 0.001$ ) (Fig. 1).

Analyzing the effect on an established biofilm, the TestCHX0.2 was most active, the cfu counts decreased in mean by 3.5 log<sub>10</sub> vs. control ( $p < 0.001$ ). Further, there were statistically significant differences between CHXcom0.2 (-2.7 log<sub>10</sub> cfu;  $p = 0.003$ ) and TestCHX0.1 (-2.4 log<sub>10</sub>;  $p = 0.012$ ) vs. control. In comparison with the CHX solutions from the pharmacy, the TestCHX0.2 was more active than CHXph0.2 ( $p = 0.005$ ) (Fig. 2A). There were no statistically significant differences in the biofilm mass (Fig. 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 TestCHX formulations vs. CHX solutions from the pharmacy; the TestCHX0.1 reduced the activity more than CHXph0.1 ( $p < 0.001$ ) (Fig. 2C).

**Tab. II** Minimal inhibitory concentrations of chlorhexidine (CHX) formulations  
Results are given related to the % of CHX within the formulations

	TestCHX0.1	TestCHX0.2	CHXcom0.2	CHXph
<i>S. gordonii</i> ATCC 10558	0.00013	0.00013	0.00013	0.00013
<i>A. naeslundii</i> ATCC 12104	0.00025	0.00013	0.00025	0.00025
<i>S. mutans</i> ATCC 25175	0.00013	0.00006	0.00013	0.00013
<i>S. sobrinus</i> ATCC 33478	0.00013	0.00013	0.0006	0.00013
<i>L. acidophilus</i> ATCC 11975	0.00025	0.0005	0.001	0.0005
<i>F. nucleatum</i> ATCC 25586	0.00025	0.00025	0.00013	0.00025
<i>P. micra</i> ATCC 33270	0.001	0.001	0.001	0.001
<i>P. gingivalis</i> ATCC 33277	0.00025	0.00025	0.00025	0.00025
<i>T. forsythia</i> ATCC 43037	0.00025	0.00025	0.00025	0.00025



**Fig. 1** 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], commercial 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

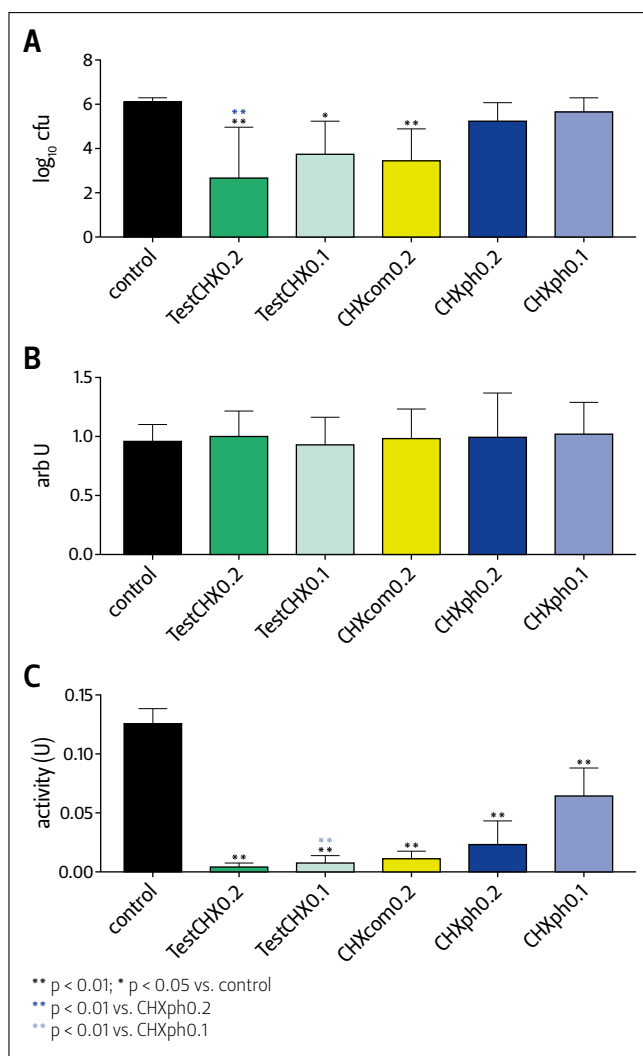
### Periodontal biofilm

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 (each  $p < 0.001$  vs. control) (Fig. 3).

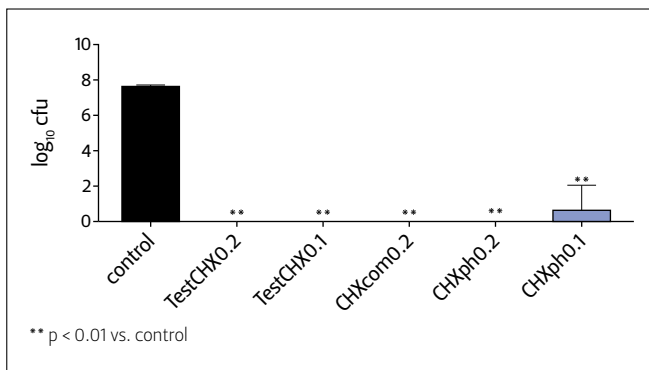
Analyzing the effect on an established biofilm, all the CHX formulations decreased statistically significantly the cfu counts. CHXcom0.2 ( $-3.9 \log_{10}$ ) was most active, followed by TestCHX0.2 ( $-3.34 \log_{10}$ ) and CHXph0.2 ( $-3.0 \log_{10}$  cfu) (each  $p < 0.001$  vs. control) (Fig. 4A). There were no statistically significant differences in the biofilm mass (Fig. 4B). Similarly, also regarding the metabolic activity, the differences were minor. Only CHXcom0.2 reduced statistically significantly the metabolic activity vs. the untreated control ( $p = 0.023$ ) (Fig. 4C).

### Viability of periodontal ligament fibroblasts after exposure to CHX formulations

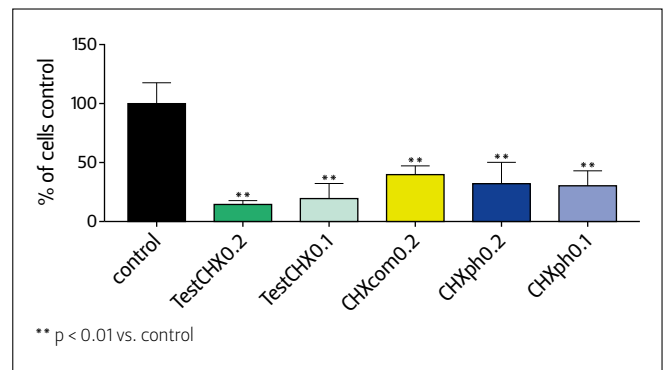
All the CHX formulations reduced statistically significantly the viability of the PDL fibroblasts vs. control. There were no statistically significant differences between the CHX preparations (Fig. 5)



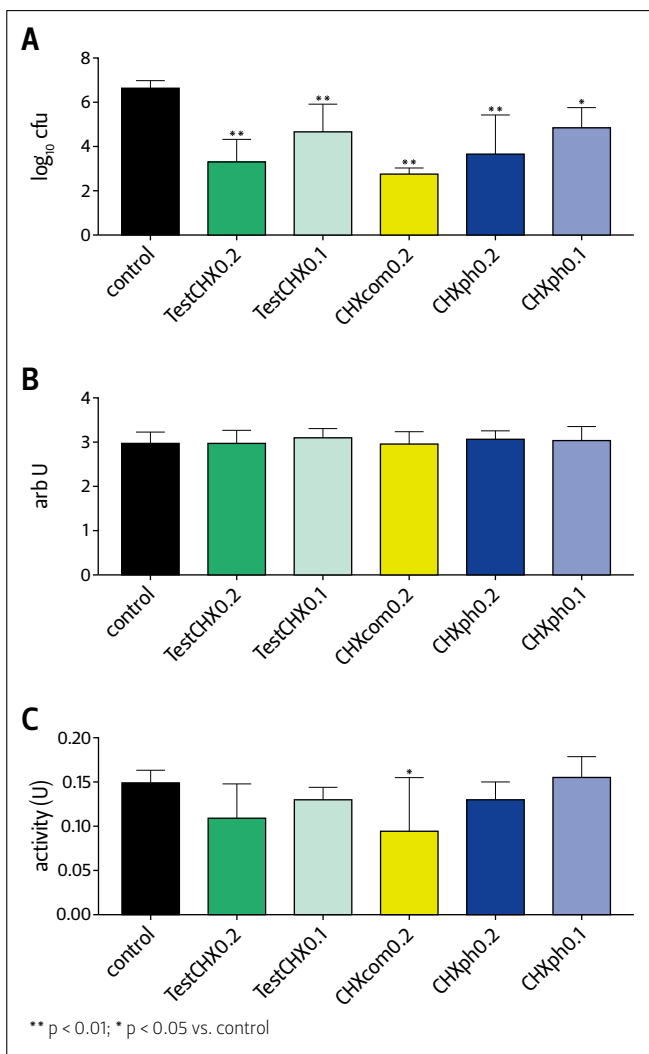
**Fig. 2** Colony-forming units (cfu) counts (A), mass (B) and metabolic activity (C) of cariogenic biofilms after culturing for 48 h and subsequent exposing to chlorhexidine formulations (test formulation 0.2% [TestCHX0.2], test formulation 0.1% [TestCHX0.1], commercial CHX formulation 0.2% [CHXcom0.2] 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.



**Fig. 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], commercial 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



**Fig. 5** 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], commercial 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.



**Fig. 4** 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], commercial 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.

## 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 the pharmacy and to the commercial product. However, the CHX-digluconate-containing formulations 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 possibly 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 denture liners (ALTINCI ET AL. 2018). Benzalkonium chloride was shown to inhibit biofilm formation of a three-species biofilm on dentine disks, the underlying mechanism discussed was as a reduced 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* spp. (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 first has to be removed mechanically. 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 close to 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  $IC_{50}$  values were 87 mg/L for CHX and 64  $\mu$ g/L for benzalkonium chloride (MULLER & KRAMER 2008). In a recent systematic review, 13 out of 51 included studies reported adverse effects of CHX mouth rinses 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

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## Zusammenfassung

### Einleitung

Karies und Parodontitis sind die häufigsten chronischen Erkrankungen beim Menschen. Beide Erkrankungen sind durch eine gestörte Homöostase verbunden mit einem Shift der Mikrobiota im dentalen Biofilm charakterisiert. Entsprechend hat die Anwendung von Antiseptika einen hohen Stellenwert. Unter den Antiseptika gilt Chlorhexidin (CHX) nach wie vor als Goldstandard. Das Ziel dieser In-vitro-Studie bestand darin, die antimikrobielle Aktivität von zwei neuen, in der Schweiz hergestellten CHX-Formulierungen zu bestimmen.

### Material und Methoden

Die zwei Testformulierungen mit 0,1% oder 0,2% CHX-Digluconat (TestCHX0.1, TestCHX0.2) wurden mit 0,1%igen und 0,2%igen CHX-Digluconat-Lösungen (CHXph0.1, CHXph0.2) ohne Zusatzstoffe und mit einem breit angewendeten kommerziell erhältlichen Produkt mit 0,2% CHX-Digluconat (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, bestehend aus fünf Bakterienspezies, und ein parodontaler Biofilm, bestehend aus sechs Bakterienspezies, kultiviert. Um die Hemmung der Biofilmbildung zu ermitteln, wurden die Substanzen in einer Konzentration von 10% der Formulierung zuerst auf die Oberflächen aufgebracht. Wenn der Biofilm zuerst gebildet wurde, erfolgte die Zugabe von 100% CHX-Formulierung für eine Minute und von 10% für eine Stunde. Die gemessenen Variablen waren koloniebildende Einheiten (KBE) (als Mass für die Zahl der Bakterien im Biofilm) und beim bereits vorhandenen Biofilm zusätzlich Stoffwechselaktivität und Biofilmquantität. Weiter wurde die Zytotoxizität der CHX-Formulierungen gegenüber parodontalen Fibroblasten bestimmt. Die statistische Auswertung erfolgte mittels ANOVA mit nachfolgendem Bonferroni.

### 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 ( $-3,5 \log_{10}$  KBE) von allen geprüften CHX-Formulierungen. Bei einem bereits vorhandenen parodontalen Biofilm wies die CHXcom0.2-Formulierung die höchste Aktivität auf ( $-3,9 \log_{10}$  KBE), gefolgt von der TestCHX0.2-Formulierung ( $-3,34 \log_{10}$  KBE) und der CHXph0.2-Lösung ( $-3,0 \log_{10}$  cfu KBE). Die CHX-Zubereitungen reduzierten die Stoffwechselaktivität im kariogenen Biofilm deutlich, nicht aber im parodontalen Biofilm. Bei beiden Biofilmen wurde die 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 ihre guten Wirksamkeit gegenüber dem kariogenen Biofilm, die im Zusammenhang mit dem Inhaltsstoff Benzalkoniumchlorid stehen könnte. Bei einem bereits vorhandenen Biofilm ist die Wirkung von CHX-Formulierungen eingeschränkt. Das unterstreicht die Notwendigkeit einer vorgängigen mechanischen Biofilmentfernung.

## Résumé

### Introduction

La carie dentaire et la parodontite sont les maladies chroniques les plus fréquentes de l'être humain. Ces deux maladies sont caractérisées par une homéostasie perturbée associée à un changement du microbiome composant le biofilm dentaire. En conséquence, le recours aux antiseptiques est d'une grande importance. Parmi les antiseptiques, la chlorhexidine (CHX) est toujours considérée comme l'étalon-or. L'objectif de cette étude in vitro était de déterminer l'activité antimicrobienne de deux nouvelles formulations de CHX produites en Suisse.

### Matériel et méthodes

Les deux formulations à tester contenant 0,1 % ou 0,2 % de digluconate de CHX (TestCHX0.1, TestCHX0.2) ont été comparées à des solutions de digluconate de CHX à 0,1 % et 0,2 % (CHXph0.1, CHXph0.2) sans additifs, et à un produit commercial largement utilisé contenant 0,2 % de digluconate de CHX (CHXcom0.2). Tout d'abord, la concentration minimale inhibitrice (CMI) des formulations de CHX sur neuf bactéries orales (associées aux caries ou à la parodontite) a été déterminée. Ensuite, l'existence d'un effet inhibiteur possible sur la formation de nouveaux biofilms ainsi que la possibilité d'influencer un biofilm existant ont été déterminées. À cette fin, un biofilm cariogène composé de cinq espèces bactériennes et un biofilm parodontal composé de six espèces bactériennes ont été cultivés. Pour déterminer l'inhibition de la formation de biofilms, les substances ont tout d'abord été appliquées sur les surfaces à une concentration de 10 % de la formulation. Lorsque le biofilm s'est formé, l'ajout de la formulation de CHX à 100 % a été

effectué pendant une minute, puis à 10 % pendant une heure. Les variables mesurées étaient les unités formant colonies (UFC, comme mesure du nombre de bactéries dans le biofilm), et en plus, pour le biofilm préexistant, l'activité métabolique et la quantité de biofilm. En outre, la cytotoxicité des formulations de CHX sur les fibroblastes parodontaux a été déterminée. L'analyse statistique a été effectuée en utilisant ANOVA suivi de Bonferroni.

### Résultats

L'activité antimicrobienne directe des formulations testées (CMI) ne différait pas de celle des formulations de comparaison. La formation du biofilm cariogène et parodontal a été bloquée par toutes les formulations, à l'exception de la solution CHX à 0,1 % sans additifs. En ce qui concerne la préexistence d'un biofilm cariogène, la formulation CHX0.2 testée a montré l'effet réducteur de germes le plus élevé ( $-3,5 \log_{10}$  UFC) de toutes les formulations CHX testées. Dans le cas d'un biofilm parodontal préexistant, la formulation CHXcom0.2 ( $-3,9 \log_{10}$  UFC) présentait l'activité la plus élevée, suivie de la formulation TestCHX0.2 ( $-3,34 \log_{10}$  UFC) et de la solution CHXph0.2 ( $-3,0 \log_{10}$  UFC). Les préparations de CHX ont réduit de manière significative l'activité métabolique dans le biofilm cariogène, mais pas dans le biofilm parodontal. La quantité des deux biofilms n'a pas été affectée par les formulations de CHX. Mais à côté de leur bonne efficacité antimicrobienne, un effet cytotoxique sur les fibroblastes parodontaux a également pu être démontré pour toutes les formulations de CHX.

### Discussion

Par rapport aux solutions de CHX sans additifs, les formulations de CHX produites en Suisse ont un effet in vitro égal ou partiellement supérieur sur les bactéries et les biofilms oraux, et leur utilisation peut donc être recommandée. À relever leur bonne efficacité contre le biofilm cariogène, qui pourrait être liée à la présence d'un ingrédient, le chlorure de benzalconium. En cas de biofilm préexistant, l'effet des formulations de CHX est limité. Cet état de fait souligne la nécessité d'une élimination mécanique préalable du biofilm.

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