ACTA ODONTOLOGICA SCANDINAVICA
2021, VOL. 79, NO. 7, 506–513
https://doi.org/10.1080/00016357.2021.1899280

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

Effect of polyhexanide as antiseptic mouth rinse against oral pathogens in an *in vitro* biofilm model

Maximilian Kollmuss[a], Konrad Tolksdorf[a,b], Sabina Noreen Wuersching[a], Reinhard Hickel[a] and Karin Christine Huth[a]

[a]Department of Conservative Dentistry and Periodontology, University Hospital, LMU Munich, Munich, Germany; [b]Department of Oral and Maxillofacial Surgery and Plastic Surgery, University Hospital, FSU Jena, Jena, Germany

ABSTRACT

Objective: The aim of this study was to evaluate the anti-biofilm effect of polyhexanide mouth rinses against oral pathogens *in vitro*.

Material and methods: Biofilms of *Candida albicans*, *Streptococcus mutans*, *Actinomyces naeslundii*, *Aggregatibacter actinomycetemcomitans*, methicillin-resistant *Staphylococcus aureus* and *Fusobacterium nucleatum* were grown on 10 mm diameter hydroxyapatite discs for 5 days. Biofilms were exposed to test substances for 30 s (ProntOral, polyhexanide 0.15%, chlorhexidine 0.2%). Another test set simulating blood contamination in the oral cavity was performed by submerging the discs in defibrinated sheep blood prior to antimicrobial exposure. Biofilm mass was determined *via* crystal violet staining. The proliferation potency of the cells after antimicrobial exposure was evaluated by plating serially diluted suspensions from extracted biofilms on agar plates and determining the number of colony-forming units (CFU/ml). Mann–Whitney-U, Kruskal–Wallis and Dunn's test were used for statistical analyses.

Results: Regardless of blood contamination ProntOral led to a significant reduction of biofilm mass in all strains. Chlorhexidine and polyhexanide reduced biofilm mass in five out of six strains and in only four strains after blood contamination. All agents significantly reduced CFU/ml from *S. mutans, A. actinomycetemcomitans* and *F. nucleatum* biofilms. *C. albicans* and *S. aureus* biofilms were only affected by ProntOral and polyhexanide. None of the antiseptics significantly reduced the CFU/ml for *A. naeslundii* biofilms. After blood contamination ProntOral and polyhexanide significantly reduced CFU/ml in all strains, whereas CHX tended to increase the CFU/ml.

Conclusions: Polyhexanide mouth rinses seem to be suitable disinfectants against oral pathogens without their anti-biofilm potential being impaired by blood.

ARTICLE HISTORY
Received 26 November 2020
Revised 15 February 2021
Accepted 2 March 2021

KEYWORDS
Polyhexanide; chlorhexidine; biofilm; mouth rinse

Introduction

During and immediately after birth, the oral mucosa of a newborn is exposed to a wide variety of microorganisms. After the initial inoculation with different bacteria, fungi and viruses, only a subset of these microorganisms is able to permanently colonize the oral cavity [1]. The physiological colonization of the oral cavity has been referred to as the oral microbiome, nurturing commensal, symbiotic and pathogenic microorganisms in an ecological equilibrium [2]. Besides their importance in oral heath, the numerous microorganisms of the oral microbiome may have a considerable effect on the immune system and health of the gastrointestinal system [3]. If there is an imbalance in the distribution of the microorganisms, allowing pathogenic bacteria and fungi to prevail, many oral diseases may occur [4]. As mechanical irritations of the oral cavity, such as brushing teeth or dental treatments can lead to a bacteraemia, the microorganisms can spread to other parts of the body and may cause further pathologies in damaged tissues such as endocarditis in predamaged heart valves [5,6].

The most common diseases of the oral cavity are caries and periodontitis, which both have an infectious aetiology associated with pathogenic bacterial biofilms [7,8]. In the field of oral and maxillofacial surgery, the osteonecrosis of the jaw as a severe complication of antiresorptive and antiangiogenic therapy and impaired wound healing are frequent clinical conditions with an infectious aetiology [9,10].

The treatment protocols for these diseases usually include the removal of the infected tissues and the disinfection of biofilm-covered surfaces [11]. As the rate of bacteraemia is significantly increased during these procedures, the application of antiseptic mouth rinses is recommended before such treatments [12,13].

Furthermore, antiseptic mouth rinses are commonly used in domestic oral hygiene for chemical plaque control, especially in cases where sufficient mechanical cleansing is not possible, for example after surgical interventions, during

CONTACT Maximilian Kollmuss maximilian.kollmuss@med.uni-muenchen.de Department of Conservative Dentistry and Periodontology, University Hospital, LMU Munich, Munich, 80336, Germany

orthodontic treatment and for patients with impaired coordination due to mental or physical disabilities [14–18].

For all of these indications, chlorhexidine (CHX) is the gold standard in dentistry and oral surgery. In many studies, CHX has proven a broad spectrum of activity against bacteria and fungi [19,20]. However, there are some side effects, especially when CHX is used over a longer period of time, including staining of the teeth and of restorations, impaired taste and irritation of the oral mucosa [21–24].

An antimicrobial agent that is used as a disinfectant in various medical fields is polyhexanide. Common indications for polyhexanide are the treatment of chronic wounds and infected total endoprostheses, but it is also used perioperatively for skin disinfection and as an intraoperative irrigation solution. However, polyhexanide can also be applied on mucous membranes and may therefore be a potential disinfectant for the oral mucosa [25–27].

As opposed to CHX, only very few side effects are reported for polyhexanide. Moreover, there are promising results regarding the proliferation of epithelial and fibroblast cells after the application of polyhexanide, which may have a positive effect on the healing process of wounds [28].

Only very few studies investigating the use of polyhexanide in the oral cavity are to be found, although commercial products are widely available. It is of great interest, if polyhexanide is a suitable alternative for CHX in oral antisepsis. Therefore, this study was conducted to evaluate if polyhexanide has the same potential as CHX against common oral pathogens in an *in vitro* biofilm model.

Material and methods

Strains and growth media

All strains were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany). Solid cultures of *Streptococcus mutans* (ATCC 251275), *Actinomyces naeslundii* (ATCC 12104), *Aggregatibacter actinomycetemcomitans* (ATCC 11123), methicillin-resistant *Staphylococcus aureus* (DSM 11822), *Candida albicans* (MYA-273) and *Fusobacterium nucleatum* (ATCC 25586) were grown on Schaedler agar plates supplemented with Vitamin K_1 and 5% Sheep Blood (BD, Franklin Lakes, NJ, USA). All microorganisms except for *F. nucleatum* were cultured at a temperature of 37° C in a facultative anaerobic atmosphere with 5.8% CO_2. The incubation of *F. nucleatum* called for obligate anaerobic conditions and was therefore performed in an anaerobic chamber (Sheldon, 5% H_2, 10% CO_2, 85% N_2). Planktonic cultures in liquid medium were prepared in Brain Heart Infusion growth medium for all strains (BHI, Becton Dickinson). For *F. nucleatum* a separate BHI growth medium was prepared under an anaerobic atmosphere.

Cultivation of Mono-species biofilms

Solid cultures of each strain were inoculated in BHI and incubated overnight. The optical density of each cell suspension was measured at a wavelength of 620 nm in a spectrophotometer (Varioskan Microplate Reader, Thermo Fisher Scientific, Waltham, MA, USA). The suspensions were diluted to an optical density of 0.1, of which 2.0 ml were added to the wells of a 48-well microtiter plate (Corning Inc., Corning, NY, USA). Mono-species biofilms were developed on the surface of artificial hydroxyapatite discs (diameter 10 mm; Clarkson Chromatography Products, South Williamsport, PA, USA) by submerging them in the diluted cell suspensions, while standing upright for maximum exposure to the planktonic microbes. The discs were incubated under the required culture conditions for five days. Prior to any further procedure, culture purity was confirmed by plating samples of the incubated suspensions on Schaedler agar plates.

Exposure of matured biofilms to antiseptic mouth rinses

The hydroxyapatite discs were washed in 0.9% sodium chloride in order to remove any planktonic cells, leaving the discs covered merely with adherent cells from the matured biofilm. The discs were then exposed to the antimicrobial agents shown in Table 1 or to 0.9% sodium chloride (control group) for 30 s, mimicking the recommended time of application for most mouth rinses. To simulate a common blood contamination of the teeth, a second test set was carried out: prior to antimicrobial treatment, the discs were submerged in defibrinated sheep blood for 30 s (Thermo Fisher, Waltham, MA, USA), leaving a thin layer of blood on the surface of the discs. For convenience sake, this test set will be referred to as 'blood group', whereas the first test set performed using only antiseptics will be referred to as 'antiseptic group'.

Quantification of residual biofilm mass

Biofilm mass was quantified by using a previously reported method [29]. The discs were rinsed in 0.9% sodium chloride, moved to clean wells, and stained by adding a 0.1% aqueous crystal violet solution to the wells. After an incubation time of 10 min at room temperature, the discs were removed from the wells, washed in sodium chloride, and dried with a clean paper towel to remove the excessive staining solution. The stained discs were then moved to clean wells and the

Table 1. Antimicrobial test agents.

Commercial name	Active substance and concentration	Abbreviation	Lot number
Dynexidin forte Kreussler Pharma, Wiesbaden, Germany	Chlorhexidine 0.2%	CHX	7L 19801
ProntOral B.Braun Medical, Sempach, Switzerland	Polyhexanide 0.15%	PO	18332M15
Polyhexanide solution Calachem Fine Chemicals, Grangemouth, UK	Polyhexanide 0.15%	PHMB	16G29-B01-330630

dye was solubilized by adding 30% acetic acid to the wells, followed by a 10 min incubation on an orbital shaker (50 rpm) at room temperature. For each condition, two replicates containing each 100 µl of the crystal violet/acetic acid solution were transferred to the wells of a clear 96-well plate. The optical density (OD) was measured at 600 nm in a spectrophotometer. Means of the two replicates were calculated and compared to the respective control group of every bacterial strain, whose OD was defined as 100%.

Biofilm extraction and quantification of viable cells

For evaluating the proliferation potency of the cells within the biofilm after antimicrobial treatment, a separate set of mono-species biofilms was grown on hydroxyapatite discs and treated with the antimicrobials in the same manner as above. The discs were either submerged in defibrinated sheep blood (blood group) or left untreated (antiseptic group) prior to exposure. The treated discs from both groups were then transferred to 15-ml falcon tubes (Corning Inc., Corning, NY, USA) containing 1.0 ml of 0.9% sodium chloride. The falcon tubes were vortexed at high intensity for 60 s to disrupt the biofilms from the surface of discs. Ten-fold dilutions of the solutions were prepared and 100 µl of each dilution were plated on Schaedler agar plates. After 48 h of incubation, the number of colony-forming units (CFUs) was counted following FDA guidelines (only plates with 25 to 250 colonies were included).

Statistics

All experiments for each condition were performed on five discs and repeated at least five times on different days. Statistical analyses were performed in Python 3.7.6 using *scipy* and *scikit* for the statistical tests and graphs were generated using *matplotlib* [30]. Box-and-whisker-plots with a logarithmic scale were used for descriptive statistics. Due to a non-normal distribution of the data determined using the Shapiro–Wilk test, subsequent inferential statistics was conducted by means of a Kruskal–Wallis test by ranks for determining stochastic dominance, followed by a Dunn's post-hoc-test with a false discovery rate correction for sub-group analysis. For evaluating the biofilm mass reduction, a Mann–Whitney-U test was used to pinpoint statistical significances between the test group and the control group. The α-level was set to 0.05 for all tests.

Results

Matured biofilms of *C. albicans, S. mutans, A. naeslundii, A. actinomycetemcomitans, S. aureus* and *F. nucleatum* were exposed to CHX, PO, PHMB and 0.9% sodium chloride for 30 s and the biofilm mass as well as the proliferation potency of the cells were determined. Residual biofilm mass was determined *via* crystal violet staining. The antiseptics affected the biofilm mass in both the antiseptic and the blood group (Table 2). The antimicrobial agents affected the proliferation potency of the microorganisms differently for each strain, measured by the number of CFU/ml after plating serially diluted biofilms. Blood contamination of the discs before being exposed to the antiseptics affected the number of CFU/ml. Box-and-whisker-plots presenting the counts of the matured biofilms after incubation with the antimicrobial test agents are shown in Figure 1 for the antiseptic group and in Figure 2 for the blood group.

Effects on the biofilm mass

The OD obtained from the crystal violet/acetic acid solution indicates that all three antimicrobials significantly reduced the biofilm mass of *C. albicans*, *A. naeslundii*, *S. aureus* and *F. nucleatum* in both the antiseptic and the blood group. PHMB did not significantly affect the amount of *S. mutans* biofilms in both groups and did not reduce the biofilm mass of *A. actinomycetemcomitans* in a blood contaminated environment. Similar results were observed for CHX, which had no influence on the *A. actinomycetemcomitans* biofilm mass in both groups and was less effective against *S. mutans* biofilms in the blood group.

Effects on cell viability and proliferation

In the antiseptic group, all antimicrobial agents significantly reduced bacterial counts from biofilms formed by *S. mutans, A. actinomycetemcomitans* and *F. nucleatum,* while biofilms of *C. albicans* and *S. aureus* were affected only by PHMB and PO, respectively. None of the tested antiseptics reduced the CFU/ml for *A. naeslundii* biofilms significantly.

In the blood group, the reduction of biofilms formed by *S. mutans*, *A. actinomycetemcomitans* and *F. nucleatum* barely differed for PO and PHMB when compared to the level of reduction achieved with each antiseptic alone. However, the submersion of the discs in sheep blood prior to application

Table 2. Reduction of biofilm mass after antimicrobial treatment of different mono-species biofilms.

	Antiseptic group			Blood group		
	CHX	PO	PHMB	CHX	PO	PHMB
C. albicans	**60.89 (8.47)**	**57.55 (14.86)**	**53.51 (16.57)**	**71.64 (9.53)**	**62.22 (16.53)**	**58.82 (14.65)**
S. mutans	**81.41 (2.59)**	**74.94 (8.42)**	96.88 (19.51)	94.88 (14.90)	**64.81 (16.01)**	92.84 (12.25)
A.actinomycetemcomitans	97.05 (14.32)	**52.80 (18.96)**	**65.94 (16.97)**	88.41 (8.74)	**76.48 (13.81)**	88.49 (18.66)
A. naeslundii	**81.05 (13.08)**	**77.12 (18.51)**	**79.76 (15.66)**	**73.15 (7.56)**	**64.77 (15.49)**	**63.20 (12.08)**
S. aureus	**70.64 (9.65)**	**77.93 (12.80)**	**71.47 (5.92)**	**73.66 (15.77)**	**71.89 (16.90)**	**72.14 (7.03)**
F. nucleatum	**51.56 (2.92)**	**55.52 (6.89)**	**43.05 (11.64)**	**69.44 (15.99)**	**81.44 (9.25)**	**75.86 (14.55)**

Biofilms grown on hydroxyapatite discs which were either submerged in defibrinated sheep blood (blood group) or left uncontaminated (antiseptic group) prior to antimicrobial exposure. Optical density obtained from crystal violet staining method was used as a parameter for quantifying the residual biofilm mass. Data presented as mean percentages of the respective control group with standard deviations. Bold font indicates statistically significant differences between the test group and the respective control group, using the Mann-Whitney-U-test for statistical analysis (α-level 0.05).

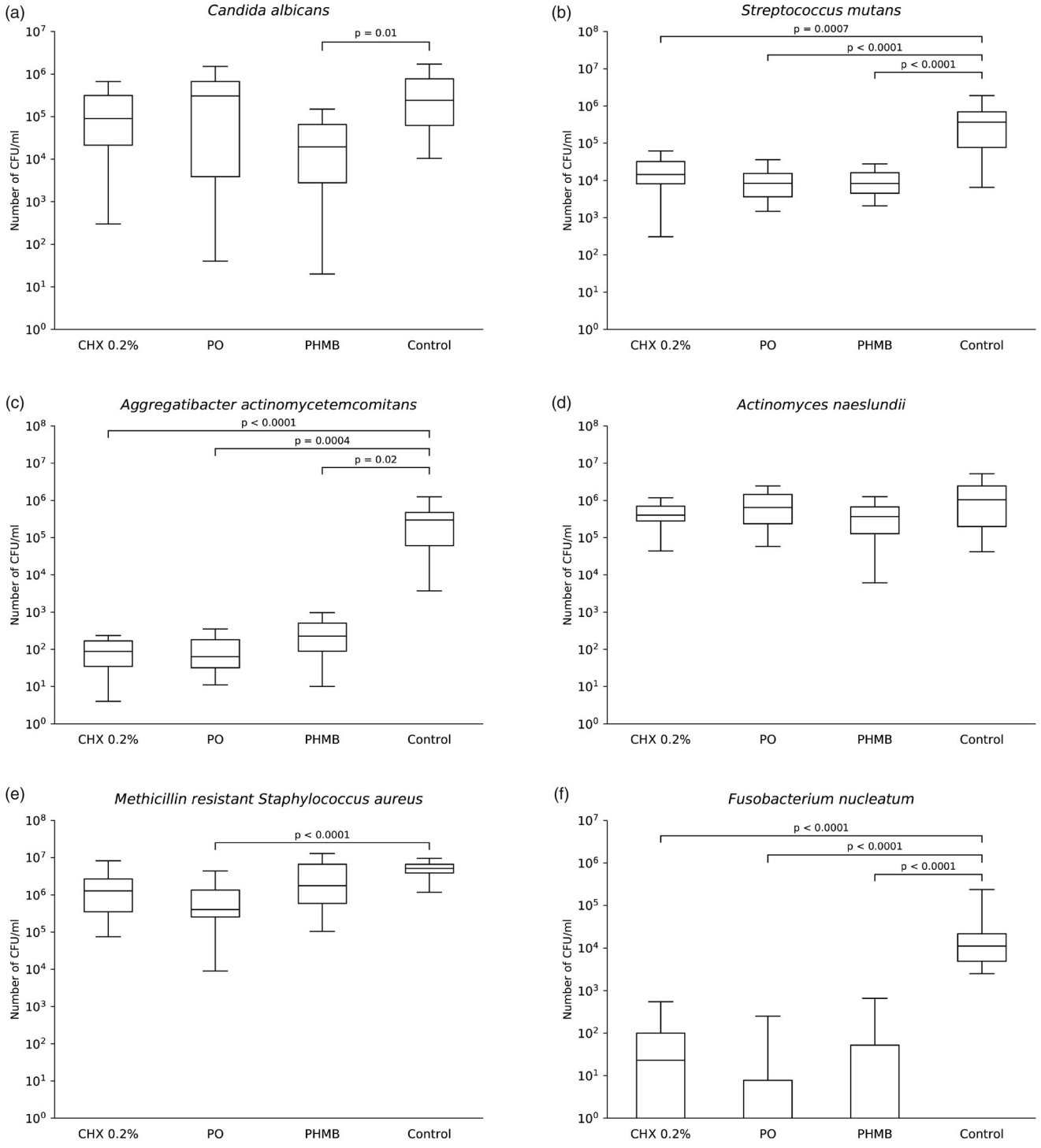

Figure 1. Reduction of CFU/ml after antimicrobial treatment shown on a log scale with a base of 10 on the y-axis, p-values from Dunn-Bonferroni-Test (α-level 0.05); (a) *C. albicans*; (b) *S. mutans*; (c) *A. actinomycetemcomitans*; (d) *A. naeslundii*; (e) methicillin-resistant *S. aureus*; (f) *F. nucleatum*.

of either PO or PHMB resulted in a significant reduction of CFU/ml for *C. albicans*, *A. naeslundii* and *S. aureus*, thereby indicating an enhanced efficacy of the antiseptics containing polyhexanide against these strains after exposure to blood. As previously observed in the antiseptic group, CHX did not significantly affect biofilms of *C. albicans, A. naeslundii* and *S. aureus* in the blood group either. Furthermore, CHX showed a similar effectiveness against *S. mutans* biofilms regardless of blood exposure. The application of CHX on blood contaminated discs increased the bacterial counts for *A. actinomycetemcomitans* and *F. nucleatum* biofilms when compared to those after application of CHX without blood contamination. Since the CFU/ml for PO and PHMB were generally lower in the blood group, further statistical differences were detected between the means of the CHX group and at least one of the polyhexanide groups for *C. albicans*, *A. actinomycetemcomitans, A. naeslundii* and *S. aureus*.

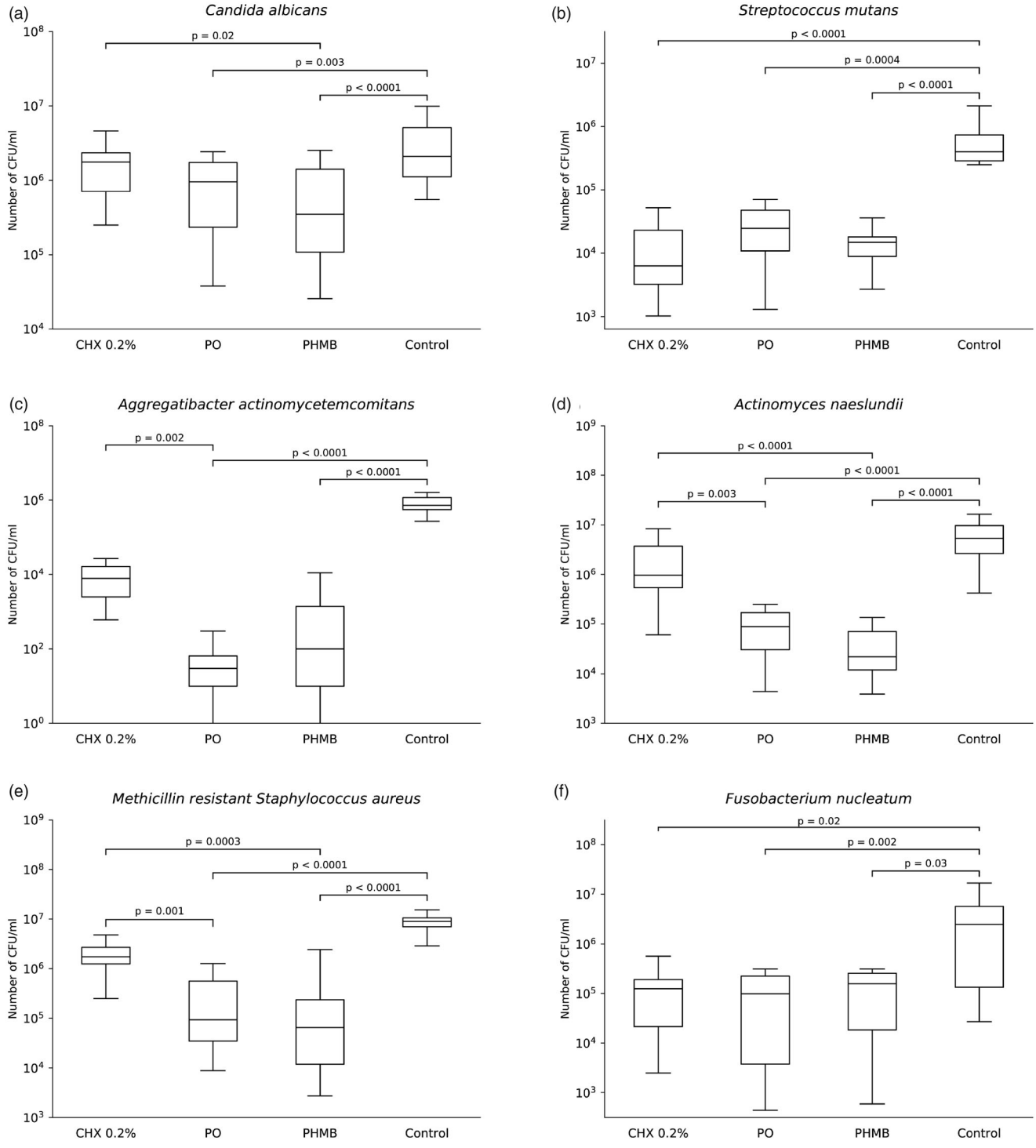

Figure 2. Reduction of CFU/ml after blood contamination with defibrinated sheep blood and antimicrobial treatment shown on a log scale with a base of 10 on the y-axis, p-values from Dunn-Bonferroni-Test (α-level 0.05); (a) *C. albicans*; (b) *S. mutans*; (c*) A. actinomycetemcomitans*; (d) *A. naeslundii*; (e) methicillin-resistant *S. aureus*; (f) *F. nucleatum*.

Discussion

The aim of this study was to evaluate the suitability of polyhexanide as an antiseptic against oral biofilms by comparing its effectiveness to the gold standard CHX. Numerous side effects that may arise during long-term application of CHX call for alternative antiseptics for chemical plaque control in the management of biofilm-associated oral diseases. Our study revealed that solutions containing polyhexanide were able to significantly reduce the microbial counts for most of the tested strains. The widely available mouth rinse solution ProntOral (PO) as well as pure polyhexanide (PHMB) therefore shows an antimicrobial potential against oral pathogens equivalent to that of CHX. We did not observe any major differences between the antimicrobial performance of PO and PHMB, as we used the same concentrations of polyhexanide (0.15%) for both formulations. Therefore, it is likely for the antimicrobial effect of PO to stem from the active ingredient

polyhexanide and not from any other ingredients of the mouth rinse such as additives for flavour and colour. Blood contamination of the antiseptics' target site is a common circumstance in the oral cavity and may influence the efficacy of the oral disinfectants. However, our results indicate that the exposure of the biofilms to blood does not lessen the antimicrobial activity of both polyhexanide formulations and in fact leads to an enhanced activity against matured biofilms for some strains. On the other hand, blood contamination triggered the tendency for CHX to reduce its antimicrobial potential.

In this study, six microorganisms were considered for the cultivation of mono-species biofilms. However, the oral cavity nurtures a vast number of species that enables the formation of complex multi-species biofilms. We chose representative pathogens for the most common infectious oral diseases in order to evaluate different potential fields of application for polyhexanide. While *A. naeslundii* and *S. mutans* are dominant pathogenic species in active caries lesions [31], *A. actinomycetemcomitans* and *F. nucleatum* are common pathogens found in subgingival plaque [32]. *C. albicans* is a key pathogen for *Candida*-associated denture stomatitis, a common fungal infection of the oral mucosa that predominantly affects immunodeficient patients wearing removable dentures [33]. As staphylococci are essential microorganisms in the aetiology of odontogenic infections alongside streptococci, *S. aureus* was included in this study [34]. In a blood contaminated environment, the level of reduction for biofilms formed by *C. albicans, A. naeslundii* and *S. aureus* was higher for polyhexanide agents compared to CHX. Similar results for *C. albicans* have been reported in a previous study investigating the effect of PHMB on *C. albicans* and other oral pathogens [35]. Since *A. naeslundii* is strongly associated with the development of cervical caries lesions in elder patients, our findings suggest that mouth rinses containing polyhexanide may be suitable alternatives to CHX in the treatment of patients with reduced compliance to good oral hygiene [17,18].

We determined the residual biofilm mass after antimicrobial treatment using crystal violet staining. In general, all antimicrobials seemed to affect the amount of biofilm mass in most strains, yet PO was the only substance that reduced the biofilm mass in all strains even in the presence of blood. For CHX and PHMB we observed a decreased potential to reduce biofilm mass of some strains in a blood contaminated environment. However, it is important to bear in mind, that staining techniques are semi-quantitative methods and have limitations that must be considered for the interpretation of the results. To be specific, crystal violet staining may overestimate the number of adherent bacteria on the stained surface, because crystal violet stains not only cells, but also any material adhering to the surface, such as components of the biofilm matrix, of which we do not know if and how they are affected by the antimicrobials [36]. Therefore, it is important to add a culture method for quantifying the cell viability, such as the determination of colony-forming units, as this is the only method providing us with information about the proliferation potency of the cells after having been exposed to the antimicrobials. Nonetheless, the significance of residual biofilm mass is not negligible, considering that in nature biofilms are not an isolated occurrence as the setup of our study may imply, but they are embedded in a dynamic biological environment. Specifically, the presence of residual biomass, regardless of its composition, facilitates the recolonization of planktonic bacteria, which are usually present at all times, thereby potentially supporting the formation of new biofilms. This indeed is a frequent problem that must be considered by clinicians when treating biofilm-associated diseases, as the mere application of any antimicrobial will not inhibit de novo recolonization by further microorganisms. In view of this, modern strategies for combatting biofilm-associated diseases include mechanical debridement combined with an antimicrobial therapy. This concept has also been advocated by clinical trials for the treatment of periodontitis, which includes a timely application of systemic antibiotics after mechanical removal of subgingival biofilms on dental surfaces [37]. Furthermore, the presence of residual biofilm mass, even if only containing matrix components or dead cells, may induce host reactions that can be crucial for the progression of the disease.

The mechanism of action of polyhexanide is similar to that of other polycationic antimicrobials such as CHX and is ascribed to interactions with the microbes' cytoplasmic membranes by binding to the negatively charged phosphate head groups of phospholipids in the bacterial cell wall. Eventually, microbial cell death is caused by membrane disruption followed by cytoplasmic shedding [38]. Numerous target sites have been reported for polyhexanide, which include the lipopolysaccharides in the outer membrane of gram-negative bacteria, the teichoic acids on the cell wall of gram-positive bacteria, peptidoglycan components of the cell wall and proteins of the cytoplasmic membrane. Up to this point, the mechanism of action of polyhexanide is very similar to that of CHX. However, recent studies propose a further, alternative mechanism for polyhexanide based on selective intracellular interactions: both gram-negative and gram-positive bacteria treated with polyhexanide display cell division arrest and chromosome condensation [39]. Polyhexanide may thereby induce bactericidal effects by DNA binding even at lower concentrations, whereas CHX requires higher concentrations in order to act bactericidal [40]. Furthermore, the anti-biofilm effect of polyhexanide is attributed to concentration-dependent binding mechanisms. While electrostatic interactions dominate at low concentrations, hydrogen bonding at higher concentrations enables the accumulation of polyhexanide in biofilm matrices, thereby creating a toxic environment for the embedded microorganisms.

In view of the possible application of polyhexanide as a perioperative antiseptic, PO and PHMB showed promising results in a blood contaminated environment against *S. aureus*, which is a key pathogen not only for odontogenic infections, but also for postoperative infections. Studies examining the cytotoxicity of CHX have revealed inhibitory effects of CHX on the growth of fibroblasts, thereby leading to impaired wound healing [41]. Polyhexanide on the other hand seems to be suitable for perioperative application, as

no such negative effect on cell proliferation has been observed [28]. Furthermore, polyhexanide causes less discolouration of the teeth as opposed to CHX, which is therefore unsuitable for a long-time application. Another disadvantage of CHX is based on its chemical structure: As CHX appears as a cation, it can easily be inactivated by anions, such as sodium dodecyl sulphate, a common detergent ingredient of toothpastes [42]. Therefore, it is important to instruct the patient about avoiding direct contact of CHX to these products. This complicates the procedure for oral hygiene and may lead to lower patient compliance. Polyhexanide on the other hand, does not show such interactions because of its polymeric chemical structure and can be therefore used immediately after the application of toothpaste.

A further aspect is the so-called substantivity, which is the ability of mouth rinse solutions to maintain their effectiveness on human tissues for an extended period of time. This property has been proven for CHX [43], however it is difficult to investigate this part in an *in-vitro* study. Therefore, it would be beneficial to conduct further *in-vivo* investigations to clarify if similar effects exist for polyhexanide.

Within the limitations of this *in-vitro* study on biofilms, polyhexanide seems to be effective against a broad spectrum of oral pathogens causing a variety of oral diseases. Simulated blood contamination does not impair the antimicrobial properties of polyhexanide. Considering the benefits of polyhexanide compared to CHX regarding wound healing and tooth discolouration, it may be a potential alternative mouth rinse solution, even for a long-time application. The antimicrobial spectrum of polyhexanide includes common caries and periodontitis pathogens as well as pathogens inducing other infections of the stomatognathic system. For a general recommendation of polyhexanide mouth rinses in dentistry and oral surgery, further studies including clinical trials are necessary.

Disclosure statement

No potential competing interest was reported by the author(s).

ORCID

Maximilian Kollmuss http://orcid.org/0000-0003-2794-6245

References

[1] Palmer C, Bik EM, DiGiulio DB, et al. Development of the human infant intestinal microbiota. PLoS Biol. 2007;5(7):e177
[2] Lederberg J, McCray AT. Ome SweetOmics – a genealogical treasury of words. Scientist. 2001;15(7):8–8.
[3] Round JL, Mazmanian SK. The gut microbiota shapes intestinal immune responses during health and disease. Nat Rev Immunol. 2009 May;9(5):313–323.
[4] Marsh PD. In sickness and in health-what does the oral microbiome mean to us? An ecological perspective. Adv Dent Res. 2018;29(1):60–65.
[5] Sreenivasan PK, Tischio-Bereski D, Fine DH. Reduction in bacteremia after brushing with a triclosan/copolymer dentifrice - a randomized clinical study. J Clin Periodontol. 2017;44(10):1020–1028.
[6] Horstkotte D. Endocarditis: epidemiology, diagnosis and treatment. Z Kardiol. 2000;89(16):IV2–11.
[7] Orland FJ, Blayney JR, Harrison RW, et al. Use of the germfree animal technic in the study of experimental dental caries. I. Basic observations on rats reared free of all microorganisms. J Dent Res. 1954;33(2):147–174.
[8] Loe H, Theilade E, Jensen SB. Experimental gingivitis in man. J Periodontol. 1965;36:177–187.
[9] Bjarnsholt T. The role of bacterial biofilms in chronic infections. APMIS Suppl. 2013(121):1–51.
[10] Sedghizadeh PP, Kumar SK, Gorur A, et al. Identification of microbial biofilms in osteonecrosis of the jaws secondary to bisphosphonate therapy. J Oral Maxillofac Surg. 2008;66(4):767–775.
[11] Quirynen M, Teughels W, De Soete M, et al. Topical antiseptics and antibiotics in the initial therapy of chronic adult periodontitis: microbiological aspects. Periodontol 2000. 2002;28:72–90.
[12] Reis LC, Rocas IN, Siqueira JF Jr, et al. Bacteremia after supragingival scaling and dental extraction: culture and molecular analyses. Oral Dis. 2018;24(4):657–663.
[13] Johnson NR, Kazoullis A, Bobinskas AM, et al. Bacterial comparison of preoperative rinsing and swabbing for oral surgery using 0.2% chlorhexidine. J Invest Clin Dent. 2015;6(3):193–196.
[14] Polat HB, Ozdemir H, Ay S. Effect of different mouth rinses on third molar surgery-related oral malodor. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2008;105(3):e1-8–e8.
[15] Cortellini P, Labriola A, Zambelli R, et al. Chlorhexidine with an anti discoloration system after periodontal flap surgery: a crossover, randomized, triple-blind clinical trial. J Clin Periodontol. 2008;35(7):614–620.
[16] Kalaga A, Addy M, Hunter B. The use of 0.2% chlorhexidine spray as an adjunct to oral hygiene and gingival health in physically and mentally handicapped adults. J Periodontol. 1989;60(7):381–385.
[17] Burtner AP, Low DW, McNeal DR, et al. Effects of chlorhexidine spray on plaque and gingival health in institutionalized persons with mental retardation. Spec Care Dentist. 1991;11(3):97–100.
[18] Clavero J, Baca P, Junco P, et al. Effects of 0.2% chlorhexidine spray applied once or twice daily on plaque accumulation and gingival inflammation in a geriatric population. J Clin Periodontol. 2003;30(9):773–777.
[19] Kollmuss M, Kist S, Obermeier K, et al. Antimicrobial effect of gaseous and aqueous ozone on caries pathogen microorganisms grown in biofilms. Am J Dent. 2014;27(3):134–138.
[20] Huth KC, Quirling M, Lenzke S, et al. Effectiveness of ozone against periodontal pathogenic microorganisms. Eur J Oral Sci. 2011;119(3):204–210.
[21] Van Strydonck DA, Slot DE, Van der Velden U, et al. Effect of a chlorhexidine mouthrinse on plaque, gingival inflammation and staining in gingivitis patients: a systematic review. J Clin Periodontol. 2012;39(11):1042–1055.
[22] Lachapelle JM. A comparison of the irritant and allergenic properties of antiseptics. Eur J Dermatol. 2014;24(1):3–9.
[23] Hepso HU, Bjornland T, Skoglund LA. Side-effects and patient acceptance of 0.2% versus 0.1% chlorhexidine used as postoperative prophylactic mouthwash. Int J Oral Maxillofac Surg. 1988;17(1):17–20.
[24] Eriksen HM, Nordbo H, Kantanen H, et al. Chemical plaque control and extrinsic tooth discoloration. A review of possible mechanisms. J Clin Periodontol. 1985;12(5):345–350.
[25] Bellingeri A, Falciani F, Traspedini P, et al. Effect of a wound cleansing solution on wound bed preparation and inflammation in chronic wounds: a single-blind RCT. J Wound Care. 2016;25(3):160–168.
[26] Eberlein T, Assadian O. Clinical use of polihexanide on acute and chronic wounds for antisepsis and decontamination. Skin Pharmacol Physiol. 2010;23:45–51.
[27] Hubner NO, Kramer A. Review on the efficacy, safety and clinical applications of polihexanide, a modern wound antiseptic. Skin Pharmacol Physiol. 2010;23:17–27.

[28] Wilkins RG, Unverdorben M. Wound cleaning and wound healing: a concise review. Adv Skin Wound Care. 2013;26(4):160–163.
[29] Wuersching SN, Huth KC, Hickel R, et al. Inhibitory effect of LL-37 and human lactoferricin on growth and biofilm formation of anaerobes associated with oral diseases. Anaerobe. 2021;67: 102301.
[30] van Rossum G, Drake F. Python 3 reference manual. Scotts Valley, CA: CreateSpace; 2019.
[31] Goeke JE, Kist S, Schubert S, et al. Sensitivity of caries pathogens to antimicrobial peptides related to caries risk. Clin Oral Invest. 2018;22(7):2519–2525.
[32] Socransky SS, Haffajee AD, Cugini MA, et al. Microbial complexes in subgingival plaque. J Clin Periodontol. 1998;25(2):134–144.
[33] Barbeau J, Seguin J, Goulet JP, et al. Reassessing the presence of *Candida albicans* in denture-related stomatitis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2003;95(1):51–59.
[34] Eckert AW, Maurer P, Wilhelms D, et al. [Bacterial spectra and antibiotics in odontogenic infections. Renaissance of the penicillins?]. Mund Kiefer Gesichtschir. 2005;9(6):377–383.
[35] Medvedec Mikic I, Cigic L, Kero D, et al. Antimicrobial effectiveness of polyhexamethylene biguanide on Enterococcus faecalis, Staphylococcus epidermidis and Candida albicans. Med Glas (Zenica). 2018;15(2):132–138.
[36] Merritt JH, Kadouri DE, O'Toole GA. Growing and analyzing static biofilms. Curr Protoc Microbiol. 2006;(1):1B.1.1-1B.1.17.
[37] Mombelli A, Almaghlouth A, Cionca N, et al. Differential benefits of amoxicillin-metronidazole in different phases of periodontal therapy in a randomized controlled crossover clinical trial. J Periodontol. 2015;86(3):367–375.
[38] de Paula GF, Netto GI, Mattoso LHC. Physical and chemical characterization of poly(hexamethylene biguanide) hydrochloride. Polymers. 2011;3(2):928–941.
[39] Chindera K, Mahato M, Sharma AK, et al. The antimicrobial polymer PHMB enters cells and selectively condenses bacterial chromosomes. Sci Rep. 2016;6:23121
[40] Karpinski TM, Szkaradkiewicz AK. Chlorhexidine–pharmaco-biological activity and application. Eur Rev Med Pharmacol Sci. 2015; 19(7):1321–1326.
[41] Liu JX, Werner J, Kirsch T, et al. Cytotoxicity evaluation of chlorhexidine gluconate on human fibroblasts, myoblasts, and osteoblasts. J Bone Joint Infect. 2018;3(4):165–172.
[42] Barkvoll P, Rolla G, Svendsen K. Interaction between chlorhexidine digluconate and sodium lauryl sulfate in vivo. J Clin Periodontol. 1989;16(9):593–595.
[43] Sodhi RN, Symington J. Penetration of chlorhexidine coating into tooth enamel: a surface analytical study. Biointerphases. 2016; 11(4):02A328.