

Cetylpyridinium chloride and miramistin as antiseptic substances in chronic wound management – prospects and limitations

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The antimicrobial activity of cetylpyridinium chloride (CPC) and miramistin (MST) solutions at different concentrations (5×10^{-5} to 0.4 %) and a dressing, containing 0.15 % CPC, were tested against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* after 30 (solutions) and 60 min (fleece) incubation, respectively. Furthermore, the cytotoxic effects of CPC and MST were examined in human keratinocyte (HaCaT) and murine fibroblast (L929) cell lines. A dose of 3×10^{-3} % CPC or MST was sufficient to entirely eradicate *S. aureus* after 30 min incubation. To achieve the same effect, higher concentrations were required against *E. coli* (0.025 % CPC; 0.0125 % MST) and *P. aeruginosa* (0.5 % CPC; 0.05 % MST). The CPC-fleece showed a high antiseptic effect against all three bacterial strains, although it did not completely eliminate *P. aeruginosa*. Both substances showed a high cytotoxic impact at higher tested concentrations (CPC $> 3 \times 10^{-3}$ %; MST $> 8 \times 10^{-4}$ %). CPC showed high antimicrobial potency at low concentrations against *S. aureus*, accompanied by low cytotoxic (side) effects at these concentrations, whilst the required minimal concentration to eradicate *E. coli* and *P. aeruginosa* was shown to be cytotoxic for keratinocytes and fibroblasts. The necessary antibacterial amounts of MST were lower, but also cytotoxic in direct contact with typical human wound cells. With regard to demographic changes and increasing bacterial resistance, new effective antiseptics, such as CPC and MST, incorporated in wound dressings without releasing an active substance could help to improve the treatment and healing rates of chronic wounds.

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INTRODUCTION

Impaired wound healing, primarily as a complication of lifestyle diseases such as diabetes, arterial hypertension and vascular disorders, is characterized by a chronically inflamed wound bed, and represents major challenges for patients and caregivers (Demidova-Rice *et al.*, 2012). As summarized in a recent report of the European Wound Management Association, the treatment of chronic wounds is a growing global problem for health-care systems and is causing increasing costs, especially in the industrialized world (Gottrup *et al.*, 2013).

An important factor for wound ‘chronification’ (i.e. progression to chronic pain), potentially followed by increased morbidity and mortality, is the bacterial colonization of wounds, which might lead to local or even systemic infection. The development of so-called biofilms is a major

issue in this context. Biofilms are polymicrobial communities that attach themselves onto wound surfaces and embed themselves in a self-produced matrix of extracellular polymeric substances, composed of DNA, proteins and polysaccharides, providing an optimal environment for bacterial cell survival. Due to their structure and organization, as well as their surrounding matrix, bacteria embedded in biofilms are significantly better protected from the effects of antiseptic (Demidova-Rice *et al.*, 2012). Although bacterial diversity is high, *Staphylococcus aureus* is represented in nearly all chronic wounds, along with further pathogens such as *Pseudomonas aeruginosa* and, especially for diabetic ulcers, *Escherichia coli* (Gottrup *et al.*, 2013). The eradication of biofilm-associated organisms with systemic antibiotics, topical antibacterial agents and antiseptics facilitates wound infection control and promotes wound healing (Demidova-Rice *et al.*, 2012).

Recently, the superiority of ‘advanced wound dressings’ compared with conventional dressings was shown in a systematic review (Heyer *et al.*, 2013). However, the results showed a high heterogeneity in study effects and routine

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Abbreviations: CPC, cetylpyridinium chloride; PHMB, polyhexamethylene-biguanide; MST, miramistin; MTT, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide.

clinical care, with an overall mean healing rate of 33% for chronic wounds. Two recent reviews also concluded that 'advanced wound dressings' had no significant advantage over conventional wound contact materials in diabetic foot ulcers (Dumville *et al.*, 2013) and venous leg ulcers (O'Meara & Martyn-St James, 2013) in terms of overall healing rates. Still, in cases of critically colonized or infected wounds, as well as biofilm formation, wound cleansing and the reduction of bacterial wound burden with antiseptics and antimicrobial wound dressings is of great importance in wound management (Daeschlein, 2013). Hence, there is still a need for the detection of new effective antiseptics as well as the development of modern wound dressings for improving chronic wound treatment in order to increase patients' health-related quality of life.

Cetylpyridinium chloride (CPC), a quaternary ammonium compound belonging to the group of cationic surface-active agents, is already known as an oral antiseptic which is used in mouthwashes, e.g. Cepacol. The antimicrobial effect of CPC results from the ability to disrupt bacterial metabolism, inhibit cell growth and induce cell death after the initial penetration of cell membranes. Binding to negatively charged bacterial surfaces is facilitated by the positive charge of CPC molecules. When used in concentrations of 0.0–0.1%, CPC is considered to be a safe antimicrobial agent for preventing biofilm formation and gingivitis according to the US Food and Drug Administration Plaque Subcommittee (Watanabe *et al.*, 2008), and shows broad-spectrum antimicrobial activity (Sreenivasan *et al.*, 2013). Thus, CPC might also represent a promising antibacterial agent for the treatment of chronic wounds.

Miramistin (MST; Fig. 1), another member of the cationic surface-active detergents, acts similarly to CPC. Although MST has been known as an effective antiseptic for the local treatment of infected wounds with low side effects since 1993 (Vasil'eva *et al.*, 1993), it is only used in Eastern Europe. Furthermore, it was identified as a potent fungicidal agent (Arzumian, 2002).

This *in vitro* study focused on these two antimicrobial substances, as they could have considerable potential in chronic wound care. Their broad spectrum of efficacy seemed to be suitable for the compound bacterial flora of a

biofilm, and thus could contribute to preventing the systemic spread of an infection originating from the chronic wound and further promote the healing process.

METHODS

Preparation of substances. In order to test the antiseptic effects, CPC powder ($C_{21}H_{38}ClN.H_2O$; CAS 6004-24-6; Sigma-Aldrich) and MST powder ($C_{26}H_{47}ClN_2O.H_2O$; Farmhim) were dissolved in 0.5% (w/v) sodium chloride to obtain a 10% (w/v) stock solution. Further dilution to produce the final test concentrations was performed with sodium chloride solution (0.5%, w/v) for antimicrobial testing and Dulbecco's modified Eagle's medium (DMEM; Biochrom) for cytotoxicity testing.

The fleece dressing to be tested consisted of an absorption layer (75% viscose, 25% polyethylene/polypropylene) and an adherence layer (100% polyethylene), and contained 0.15% CPC (Kemex).

Test organisms and nutrient solutions. The following bacterial strains were used for susceptibility tests: *S. aureus* (DSM-799), *P. aeruginosa* (DSM-939) and *E. coli* (DSM-11250) (German Collection of Microorganisms and Cell Cultures/Deutsche Sammlung von Mikroorganismen und Zellkulturen). All cultures were maintained on casein/soy peptone agar [CSA; 1.5% (w/v) agar in casein/soy peptone broth (CSB); AppliChem].

Test suspensions for each organism were prepared from one fresh colony each in CSB containing 1.5% (w/v) casein peptone, 0.5% (w/v) soy peptone, 0.5% (w/v) sodium chloride and 50 ml double-distilled H_2O . pH was adjusted to 7.2 using 5 M sodium hydroxide (AppliChem) and the mixture was incubated overnight at 37 °C under aerobic conditions.

Antimicrobial activity.

Quantitative suspension method. A prior published quantitative suspension method (Koburger *et al.*, 2010), based on DIN EN 1040 (Deutsches Institut für Normung, 2005a) and DIN EN 1275 (Deutsches Institut für Normung, 2005b), was adapted and modified to test CPC and MST solutions at 14 different concentrations with regard to their antiseptic effect against the three test organisms, without any organic load. For the investigations, serial 10-fold dilutions of the bacterial test suspension were prepared in CSB and inoculated with test solutions (CPC and MST) to produce the desired final concentrations ($0.4\text{--}5 \times 10^{-5}\%$). After 30 min incubation at room temperature, aliquots (100 μ l) were spread onto nutrient agar plates and incubated overnight under aerobic conditions at 37 °C. Surviving bacteria (c.f.u. ml^{-1}) were counted with an eCount Colony Counter Pen (VWR). Each analysis was performed in triplicate.

Dressing test via culture broth method. The CPC-fleece was tested against the three bacterial strains in a modified test procedure based on a previously published culture broth method (Thomas & McCubbin, 2003; Wright *et al.*, 1999) and ISO EN 20743 (International Organization for Standardization, 2012), evaluating its direct impact on the microbes, as well as the efficacy of the released CPC only. Pieces of area 1 cm^2 were prepared in an aseptic manner using sterile scissors. Two test series of 10 fleece samples each were set up in multi-well plates (Sarstedt). A bacterial test suspension was prepared as described above and serially 10-fold diluted in CSB.

The samples of the first test series were pre-treated with 80 μ l CSB (maximum absorption quantity; evaluated in pre-tests), and the plate was sealed with Parafilm and incubated for 3 h at room temperature in order to extract CPC from the fleece. Subsequently, the samples were recovered from the plate and separately placed in sterile filters (100 μ m pores; Partec) within sterile vials and centrifuged at 1500

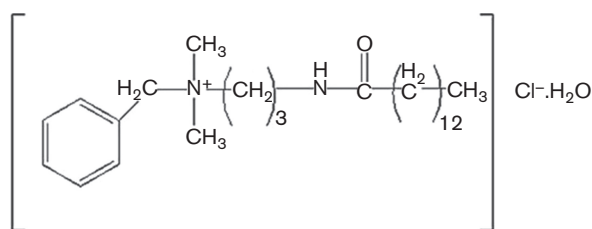


Fig. 1. Chemical structure of MST. Modified from a data sheet of Farmhim (Shostka City, Ukraine).

r.p.m. (400 × g) for 10 min to extract the CPC-containing fluid from the fleece. Aliquots of these fluids and dilution steps (50 µl each) were mixed and incubated at 37 °C for 60 min.

For the second test series, the serial dilution steps were mixed again 1 : 1 with CSB to obtain equal bacterial concentrations ($\sim 1 \times 10^8$ c.f.u. ml⁻¹) for both test series. Aliquots (80 µl) of this mixture were applied to the fleece, repeating the procedures of sealing the plate and incubation for 60 min at 37 °C. After incubation, the second test set was recovered in the same manner as the first to obtain the fluid holding the surviving bacteria from the fleece. Both series were then spread onto agar plates and incubated overnight at 37 °C. Bacterial counts (c.f.u. ml⁻¹) were again carried out with an eCount Colony Counter Pen (VWR) and experiments were performed in triplicates.

For both the suspension and fleece investigations, initial bacterial c.f.u. counts ($\sim 1 \times 10^8$ ml⁻¹) were determined by spreading untreated culture controls of each experiment onto agar plates to allow exact calculations of surviving organisms as well as reduction rates against this control.

Cytotoxicity of CPC and MST. L929 (murine fibroblast) and HaCaT (human keratinocyte) cell lines (LGC Standards) were cultivated in DMEM (Biochrom) supplemented with 10% (v/v) FBS (Sigma-Aldrich), 1% (w/v) HEPES and 1% (v/v) penicillin/streptomycin (Biochrom) in 5% CO₂ at 37 °C.

In order to test the cytotoxicity of CPC and MST, cells were trypsinated (trypsin/EDTA; Biochrom), counted (NucleoCounter; Chemometec), diluted to 200 000 cells ml⁻¹, seeded to a density of 20 000 cells per well in 96-well plates (Sarstedt) and cultivated overnight in 5% CO₂ at 37 °C. Cell culture medium was then replaced by DMEM with eight different concentrations of either CPC or MST. The percentage of surviving cells was detected via the MTT test [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide; Sigma] after 15 and 60 min incubation in accordance with the manufacturer's instructions and determined photometrically at 570 nm. Cytotoxicity was judged according to the established cytotoxicity scale of the Fraunhofer Institute for Interfacial Engineering and Biotechnology (IGB, Stuttgart). In this scale, the survival rate of proliferating cells is ranked referring to the positive control as 100%. A proliferation rate >81% is considered non-cytotoxic, whilst a value <61% is considered highly cytotoxic. A moderate or weak cytotoxicity is designated by 61–70% or 71–80%, respectively.

CPC release from the fleece. HPLC was performed by an external laboratory (Analytical Services) for the quantification of CPC release. Briefly, 1 cm² pieces of fleece were placed in suitable well plates and inoculated with 0.9% sodium chloride solution (80 µl). Plates were sealed with Parafilm and incubated at room temperature for 60 min and 6 h, and fluids were subsequently extracted and analysed. A calibration curve for CPC was established from different concentrations of a standard CPC stock solution. Values of release were calculated against this curve and results expressed as µg (cm² fleece)⁻¹.

Statistical analysis. Bactericidal activity was indicated by a reduction of bacterial counts [\log_{10} (c.f.u. ml⁻¹)] and defined as none for <0.5, low for 0.5–1, moderate for >1 but ≤3 and high for >3 log reduction, according to previously used assessment scores (Gallant-Behm *et al.*, 2005; Wiegand *et al.*, 2009).

Significant differences of the *in vitro* antiseptic effects of CPC and MST as well as of the CPC-fleece compared with CPC release from fleece were assessed by Student's *t*-test with the SPSS Statistics package (IBM). Mean ± SD values were calculated from triplicates. Differences were considered statistically significant at $P < 0.05$.

In detail, a one-sided *t*-test was used for the evaluation of bacterial reduction by CPC or MST against the positive control, and a two-sided *t*-test for differences between the antimicrobial efficacy of CPC-fleece and CPC release.

RESULTS

Bacteriotoxicity of CPC and MST

Minimum concentrations of 6×10^{-3} % CPC and 3×10^{-3} % MST were required to completely eradicate *S. aureus* after 30 min incubation (Figs 2 and 3). To eradicate *P. aeruginosa*, 0.05% MST was necessary, whilst the highest tested concentration of CPC (0.4%) was able to halve the initial bacterial counts. *E. coli* was eradicated at 0.025% CPC or 0.0125% MST. The minimum concentrations of CPC that reduced bacteria significantly ($P < 0.05$) were 2×10^{-4} % for *S. aureus*, 1.5×10^{-3} % for *E. coli* and 0.05% for *P. aeruginosa*. For MST, minimum concentrations of 2×10^{-4} , 5×10^{-5} and 3×10^{-3} % were required for a significant reduction of *S. aureus*, *E. coli* and *P. aeruginosa*, respectively.

Antimicrobial efficacy testing of CPC fleece and CPC release

The fleece showed a high antiseptic effect for *S. aureus* [$7.34 \pm 0.63 \log_{10}$ (c.f.u. ml⁻¹)] as well as for *E. coli* [$5.04 \pm 0.23 \log_{10}$ (c.f.u. ml⁻¹)], whilst a moderate reduction [$2.39 \pm 0.24 \log_{10}$ (c.f.u. ml⁻¹)] could be observed for *P. aeruginosa* (Fig. 4). These effects were significant ($P < 0.05$) for all three bacterial strains.

In comparison, released CPC exhibited only moderate [$2.90 \pm 0.48 \log_{10}$ (c.f.u. ml⁻¹)] activity for *S. aureus*, whilst displaying low [$1.00 \pm 0.56 \log_{10}$ (c.f.u. ml⁻¹)] and even no [$0.06 \pm 0.10 \log_{10}$ (c.f.u. ml⁻¹)] effects for *E. coli* and *P. aeruginosa*, respectively. Significant reductions were only seen for *S. aureus* and *E. coli*.

CPC release from the fleece of 0.496 and 0.969 µg cm⁻² was detected after 60 min and 6 h, respectively.

Cytotoxicity of CPC and MST

Concentrations of 3×10^{-3} % and higher for CPC and MST resulted in sedimentation in cell culture medium, influencing the photometric measurement. Hence, only the supernatant of samples and controls was measured photometrically to determine the survival rate of cells. CPC and MST showed severe cytotoxic effects even at low concentrations, whereby the cytotoxicity of MST was stronger than that of CPC (Figs 5 and 6). Concentrations $< 3 \times 10^{-3}$ % for CPC or $< 8 \times 10^{-4}$ % for MST were determined to be non-cytotoxic according to the assessment scores applied.

DISCUSSION

Antiseptic efficacy of CPC

The *in vitro* antimicrobial efficacy of the cationic detergent CPC has been known since the 1940s. It is established in mouthwashes to reduce a broad spectrum of oral bacteria (Sreenivasan *et al.*, 2013). Its effective antimicrobial activity was proven against *S. aureus* in an *in vitro* study (Watanabe

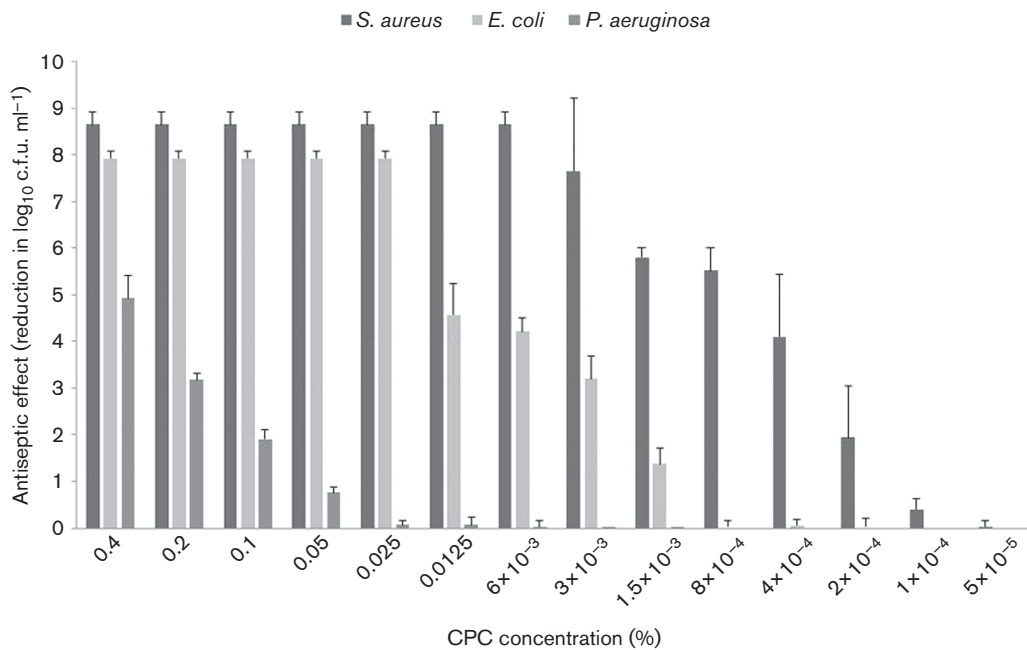


Fig. 2. Antiseptic effects of CPC at different concentrations against *S. aureus*, *E. coli* and *P. aeruginosa* after 30 min incubation. Values are expressed as mean \pm SD reduction in log₁₀ c.f.u. ml⁻¹ of three experiments. The minimum concentrations of CPC that reduced bacteria significantly (one-sided *t*-test, $P < 0.05$) were 2×10^{-4} % for *S. aureus*, 1.5×10^{-3} % for *E. coli* and 0.05 % for *P. aeruginosa*.

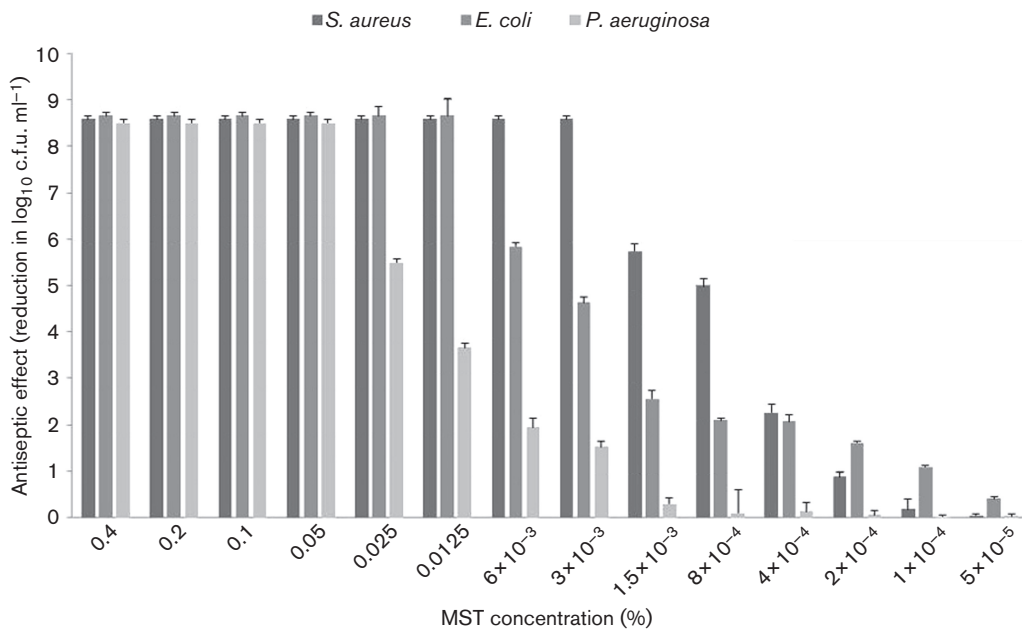


Fig. 3. Antiseptic effects of MST at different concentrations against *S. aureus*, *E. coli* and *P. aeruginosa* after 30 min incubation. Values are expressed as mean \pm SD reduction in log₁₀(c.f.u. ml⁻¹) of three experiments. The minimum concentrations of MST that reduced bacteria significantly (one-sided *t*-test, $P < 0.05$) were 2×10^{-4} % for *S. aureus*, 5×10^{-5} % for *E. coli* and 3×10^{-3} % for *P. aeruginosa*.

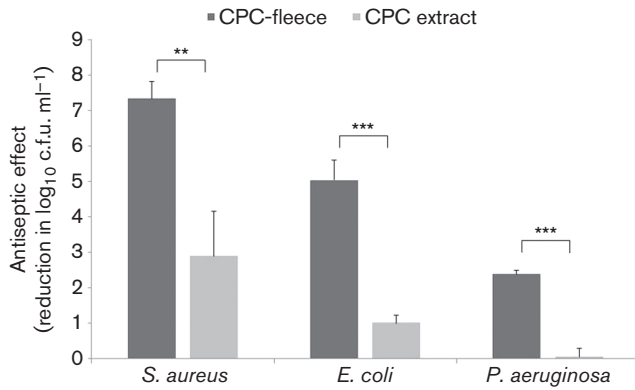


Fig. 4. Antiseptic effects of a 0.15% CPC-containing fleece and released extract from fleece against *S. aureus*, *E. coli* and *P. aeruginosa* after 60 min incubation. Values are expressed as mean \pm SD reduction in log₁₀ c.f.u. ml⁻¹ of three experiments. Differences between the antimicrobial efficiency of CPC-fleece and CPC release from fleece were tested with a two-sided *t*-test (** $P < 0.01$; *** $P < 0.001$).

et al., 2008), as well as against *Campylobacter jejuni* (>6 log reduction), as a disinfectant (Gutiérrez-Martín *et al.*, 2011). Additional antifungal effects of CPC mouthwashes were reported recently (Fu *et al.*, 2014). The present study confirmed this high antimicrobial efficacy of CPC against *S. aureus* even at dilutions up to 3×10^{-3} % after only 30 min of exposure. As expected, higher concentrations of CPC were necessary to eliminate the more resistant strains of *E. coli* (0.025 %) and *P. aeruginosa* (>0.4 %). Unfortunately, these concentrations revealed a distinct cytotoxic effect on fibroblasts and keratinocytes *in vitro*. Presumably, extension of the application time may increase the antiseptic effect and possibly lower the required concentration of CPC.

The antiseptic potency of the CPC-fleece (0.15 %) was shown by an effective reduction of the tested bacteria. Its efficacy correlated with the results of the quantitative suspension method: *S. aureus* and *E. coli* were almost completely eradicated. As for *P. aeruginosa*, a major proportion was eliminated. Part of the reason for its good antiseptic effect seemed to be the release of CPC from the fleece, as a certain amount of emerging substance could be detected after 60 min. Nevertheless, the main impact on the bacterial test strains could be seen in the cases of full contact with the dressing over 60 min. The most likely reason for this may be the fact that only a small proportion of CPC is released, which is not enough to exhibit complete antibacterial activity. Still, it has to be assumed that the released CPC not only displays a positive effect on diminishing bacterial counts, but also negative side effects on wound cells. A recent study of a mouthwash containing 0.07 % CPC over a follow-up period of 6 months with daily usage in 35 patients reported a significant reduction of oral bacteria as well as increased healing rates for gingivitis without relevant side effects (Costa *et al.*, 2013). However, when transferred to the situation of an infected and inflamed wound bed, the relevance of the cytotoxic effects seen in this study need to be discussed further, especially in the context of adverse effects and the benefits of a dressing composition that is not releasing its embedded substance into the wound. As stated above, a certain amount of the embedded CPC was released from the dressing, exhibiting an antibacterial activity on its own (Fig. 4). As adverse effects due to cytotoxicity of CPC should be reduced to a minimum in order not to interfere with cell proliferation and formation of granulation tissue, the general release of active substances from a wound dressing should be minimized or, better, completely prevented. Thereby, the embedded active agent would exert its antibacterial effect on the bacteria present on the wound surface and in the absorbed contaminated wound fluid, rather than disturbing healing

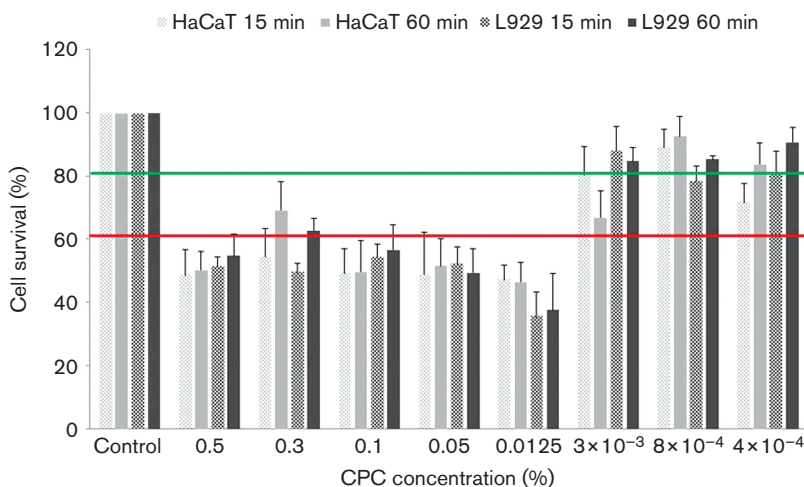


Fig. 5. Cytotoxic effects of CPC at different concentrations on L929 murine fibroblast and human HaCaT cell lines after 15 and 60 min incubation visualized as cell survival rate (%). Evaluated according to the cytotoxicity scale of the Fraunhofer Institute for Interfacial Engineering and Biotechnology. A proliferation rate >81 % (green line) is considered non-cytotoxic and a proliferation rate <61 % (red line) is considered highly cytotoxic. The positive control is designated 100 %. Values are expressed as mean \pm SD.

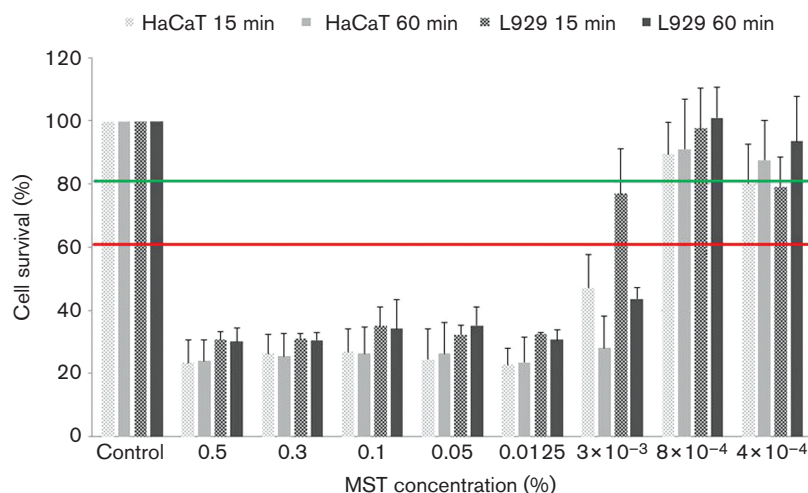


Fig. 6. Cytotoxic effects of MST at different concentrations on L929 murine fibroblast and human HaCaT cell lines after 15 and 60 min incubation visualized as cell survival rate (%). Evaluated according to the cytotoxicity scale of the Fraunhofer Institute for Interfacial Engineering and Biotechnology. A proliferation rate $>81\%$ (green line) is considered non-cytotoxic and a proliferation rate $<61\%$ (red line) is considered highly cytotoxic. The positive control is designated 100%. Values are expressed as mean \pm SD.

processes due to potential cytotoxic side effects of the substances released into the wound bed.

Antiseptic efficacy of MST

MST has been known in Eastern Europe since 1993 as a potent bactericidal (Vasil'eva *et al.*, 1993), antiviral (Agafonov *et al.*, 2005a, b) and fungicidal agent (Arzumanian, 2002) for local and superficial (wound) treatment with only few side effects. *In vitro*, 0.01% MST eliminated streptococci after 60 min and Gram-negative cocci after 6 h of exposure. In clinical practice, the results for nine out of 10 patients were satisfying with regard to the antiseptic potency of MST in the local treatment of purulent inflammatory infections for 14 days. It has to be emphasized that no negative side effects were recorded (Vasil'eva *et al.*, 1993). These results appear to contradict those of the presented study: MST showed high cytotoxic effects ($\geq 8 \times 10^{-4}\%$) *in vitro*, even exceeding those of CPC ($\geq 3 \times 10^{-3}\%$). Thus, the effect of MTT on cells involved in the wound-healing process should be compromising according to these results, rather than stimulating. Therefore, it has to be presumed that in the complex environment of a chronic wound, MST exerts a stronger or merely earlier cytotoxic impact on the microbiological burden rather than the more slowly proliferating cells of the human organism.

The possibility of drawing final conclusions regarding the actual cytotoxic effects of CPC and MST within the complex microenvironment of the healing wound is limited in the present study. Further studies, such as co-culture models or 3D cell cultures in combination with bacterial suspensions as well as animal models and *in vivo* assessments, will be necessary to further investigate and evaluate the actual cytotoxic impact.

Potential use of CPC and MST

Due to demographic changes, the incidence of chronic diseases such as diabetes mellitus type II, arteriosclerosis

and chronic venous insufficiency is increasing, leading to recurrent skin lesions especially in the lower limbs, such as diabetic foot ulcers and venous ulcers (Sen *et al.*, 2009). Long-standing morbidity, amputation and mortality could result. This necessitates the enhanced local treatment of wounds to improve quality of life. Not only light and well-fitting wound coverage, but also advanced wound dressings containing antiseptic agents such as silver or polyhexamethylene biguanide (PHMB) have been developed to handle infection and support the healing process. The treatment of chronic wounds has been visibly ameliorated in recent years by those formulations (Heyer *et al.*, 2013). For instance, ionic-silver-containing alginate dressings demonstrated broad-spectrum *in vitro* activity against *Candida albicans*, *E. coli*, *P. aeruginosa*, *S. aureus*, β -haemolytic *Streptococcus* and strictly anaerobic bacteria (Hooper *et al.*, 2012), and good clinical outcomes were described for carboxymethyl cellulose dressings with ionic silver (Beele *et al.*, 2010). In contrast, a systematic review of the Cochrane Collaboration comparing silver-containing dressings against non-medicated dressings concluded that 'there is insufficient evidence to establish whether silver-containing dressings or topical agents promote wound healing or prevent wound infection' (Storm-Versloot *et al.*, 2010). Polyurethane foam or biocellulose wound dressings containing 0.5% PHMB were even more effective in reducing pain and bacterial burden in infected acute and chronic wounds compared with silver dressings (Eberlein & Assadian, 2010; Eberlein *et al.*, 2012).

In contrast to systemic antibiotic treatment, topical application of antiseptics offers a number of advantages, e.g. prevention of systemic adverse effects, decreased induction of bacterial resistance and higher concentration of the antibacterial agent at the site of infection (Lio & Kaye, 2004). This was revealed by pexiganan. In a multi-centre trial of a cream containing this synthetic antimicrobial peptide for treating mildly infected diabetic foot ulcers, the authors stated that topical pexiganan was an effective alternative to oral antibiotic therapy and offered

the opportunity to reduce the risk of selecting antimicrobial-resistant bacteria (Lamb & Wiseman, 1998; Lipsky *et al.*, 2008). However, these antimicrobial peptides, so-called chemical-produced novel antibiotics, have been considered to compromise natural immunity (Habets & Brockhurst, 2012). Based on the fast emergence of *S. aureus* strains resistant against the structure of these peptides, these strains also rapidly developed cross-resistance to a peptide produced by the human immune system.

Despite the benefits of antiseptic agents in topical chronic wound treatment, the increasing emergence of bacterial resistance against them has to be considered (Sütterlin *et al.*, 2012). It is important that new or rarely used antiseptic agents are tested to develop a wide range of options for chronic wound management. This was the purpose of testing CPC and MST. The latter was already approved for human usage in Russia, even though with a different indication. Both showed high antiseptic potential, but also a certain degree of cytotoxicity on the main cells of wound healing (fibroblasts and keratinocytes). The amounts of MST required to eliminate all three tested bacterial strains were 10 times lower than those of CPC. Therefore, it should be preferred due to its higher antimicrobial efficacy at the same level of negative effects on wound cells. *In vivo* tests are needed to confirm these observations. Otherwise, by creating a wound dressing like the above-mentioned PHMB foam, which does not release its active substance into the wound (unpublished data), a possible cytotoxic potential could be neglected, as explained above. In this case, a lower effect has to be expected. However, with PHMB foam a local improvement of the wound situation was achieved by absorption of the infectious wound fluid and, therefore, systemic infection (sepsis) could be avoided.

Although CPC and MST demonstrated high antimicrobial efficacy in this *in vitro* study, there are several important caveats with regard to this conclusion. The substances may show a reduced *in vivo* antiseptic activity caused by the environment of a (chronic) wound. Inflammatory mediators, degrading enzymes (e.g. matrix metalloproteinases), increased protein levels, as well as varying pH values could diminish and limit their activity. Further research regarding these possible interactions of antimicrobial substances and factors influencing the wound environment needs to be conducted in the future.

CONCLUSION

CPC and MST showed high antimicrobial activity with low cytotoxic side effects against *S. aureus* at low concentrations. Both substances could eradicate *E. coli*; however, only MST was able to eradicate *P. aeruginosa* at the concentrations tested. The necessary antimicrobial concentrations of the tested substances were found to be cytotoxic for L929 fibroblast and HaCaT keratinocyte cell lines *in vitro*. An extended incubation time (>30 min) is

considered to raise the antiseptic activity of CPC and MST at lower cytotoxicity levels. As proven for PHMB, there is a possibility of embedding substances in wound dressings without releasing the agent. This might be a potential option to reduce or even avoid cytotoxic side effects which are tolerated so far in dressings releasing antimicrobial agents in the wound bed. With a view to demographic changes and rising resistance of bacteria, new effective antiseptics, such as CPC and MST, incorporated in wound dressings could help to improve the treatment and healing rates of chronic wounds.

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