Protective effect of polihexanide on HaCaT keratinocytes in co-culture with Staphylococcus aureus

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Introduction
Staphylococcus aureus is one of the most important pathogens of nosocomial infections and is a common complication during the treatment of chronic wounds. It can exhibit a range of antibiotic resistancy (MRSA, Methicillin-resistant Staphylococcus aureus). Polihexanide is regarded first choice for the antimicrobial treatment of critical colonized or infected chronic wounds because of its good skin tolerance beside its antimicrobial effects. It possesses a specific mechanism of action against acidic lipids of the bacterial membrane and has only little effect on the neutral lipids of the human cell membrane [1]. In fact, Kramer et al. showed that polihexanide promotes wound healing in an animal model [2]. Furthermore, we have investigated the effect of polihexanide on human keratinocytes and found that polihexanide in low concentrations stimulates cell proliferation [3]. Hence, we have used a co-culture system of HaCaT keratinocytes and Staphylococcus aureus to test the capacity of polihexanide to protect the cells from the bacterial damage.

Material & Methods
HaCaT keratinocytes were cultured with increasing concentrations of Staphylococcus aureus and with or without the addition of polihexanide (PHMB) as well as the extract of a HydroBalanced wound dressing with PHMB (Suprasorb® X+PHMB, Lohmann & Rauscher). Viability and proliferation of HaCaT keratinocytes was investigated by means of the ATPlite™ kit (Perkin Elmer). Viable Staphylococcus aureus cells were quantified via staining with SYTO-9 (Molecular Probes).

Results
Staphylococcus aureus had a concentration-dependent negative effect on HaCaT cell viability and proliferation (fig. 1). The addition of polihexanide (1 µg/ml) prevented damage to the HaCaT cells and restored normal cell proliferation (fig. 2). In accordance, the addition of 0.4 µg/ml and 1 µg/ml of polihexanide, respectively, reduced the number of viable bacterial cells as determined via SYTO-9 staining (fig. 3). Because polihexanide is often used in wound dressings we have investigated the effect of an extract of the HydroBalanced wound dressing with PHMB in our co-culture system and observed a significant reduction of Staphylococcus aureus growth (data not shown). Hence, the extract of the HydroBalanced wound dressing with PHMB was able to protect the keratinocytes from bacterial damage and, furthermore, had a positive influence on the cell proliferation (fig. 4).

Conclusions
Polihexanide is a highly potent antimicrobial agent, which possesses low cytotoxicity and very good skin tolerance. In addition, it is able to induce cell proliferation in vitro [3] as well as in an animal model [2]. Therefore, polihexanide seems to be an ideal antimicrobial substance in wound dressings for treating chronic wounds. Furthermore, we have been able to prove the antimicrobial activity of polihexanide and the HydroBalanced wound dressing with PHMB in a co-culture of HaCaT keratinocytes and Staphylococcus aureus in vitro. It protects the cells from the bacterial damage and allows normal cell growth.

References