Viability and proliferation of fibroblasts, keratinocytes and HaCaT-cells influenced by polihexanide

C. Wiegand¹, M. Abel², A. Kramer³, G. Müller³, P. Ruth², U.-C. Hippler³
¹Department of Dermatology, University Medical Center Jena, Germany
²Lohmann & Rauscher GmbH & Co. KG, Rengsdorf, Germany
³Institute for Hygiene and Environmental Medicine, Greifswald, Germany

Introduction
Wound dressings combined with antimicrobial agents are increasingly utilized in the treatment of critical colonized or infected chronic wounds. Povidone-iodine and octenidine are considered more or less equivalent for disinfecting acute wounds, whereas polihexanide is regarded first choice for chronic wounds because of its good skin tolerance beside its antimicrobial effects. Furthermore, a positive influence of polihexanide on wound closure was observed in individual clinical cases. Therefore we investigated the influence of polihexanide on viability and proliferation of three skin connected cell lines.

Material & Methods
Viability and proliferation of normal human dermal fibroblasts (NHDF), normal human epidermal keratinocytes (NHEK) and HaCaT-cells (human adult high calcium low temperature keratinocytes) were investigated via microscopic evaluation of live and dead cells (analySIS® 3.1 Soft Imaging System GmbH, Germany) and by means of the ATPLite™ M kit (Perkin Elmer). To distinguish between live and dead the cells were stained with SYTO-13 and Ethidiumhomodimer-2 (Molecular Probes). The luminometric ATP assay is based on the detection of light generated by the ATP dependent enzymatic conversion of D-luciferin by luciferase.

Results
The influence of polihexanide on cell proliferation, investigated via measurement of ATP content, is shown in fig. 1. Polihexanide in concentrations of 0.2 - 2 µg/ml has a significant proliferative effect on keratinocytes. In higher concentrations (> 2 µg/ml polihexanide) a dose-dependent decrease of the cell proliferation was observed. The results of the ATP assay were supported by microscopic evaluation of the area covered by cells after staining with SYTO-13 and EthD-2 (fig. 2). The results suggest three possible concentration-dependent effects of polihexanide on cells: proliferative, anti-proliferative but not harmful and cytotoxic (fig. 3 and fig. 4).

Conclusions
Polihexanide seems to be an ideal antimicrobial substance in wound dressings for treating chronic wounds because of its low cytotoxicity, good skin tolerance and positive influence on proliferation. Thus, a significant increase of the proliferation of human keratinocytes and HaCaT-cells by polihexanide was found. In higher concentrations a dose-dependent decrease of foremost proliferation and then cell viability was observed. These observations are in accordance with in vivo studies published Kramer et al. 2004. There the authors were able to show that application of polihexanide significantly shortened the time till wound closure [1].

References