

# Synergistic effects of tissue tolerable plasma and polihexanide to promote healing of chronic wounds - *in vivo* and *in vitro* results

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## Résumé

The effects of combined plasma - polihexanide (PHMB) application on cell integrity, cytotoxicity and its irritative and inflammatory potential were tested *in vitro* and *in vivo* in two dogs. The combined application showed synergistic effects in the treatment of chronic wounds. Tissue tolerable plasma works as promoter for wound healing and can be beneficially combined with the antiseptic PHMB polihexanide to avoid bacterial recolonization.

## Introduction

A chronic wound is characterized by a chronic inflammation and critical bacterial colonization which prevent it from healing in an orderly set of stages. Only a small number of planctonic bacteria are sufficient to adhere to the wound surface; to multiply and to develop into microcolonies over short time, which in turn form larger aggregates known as biofilms [1, 2]. In this context, two therapeutic problems have to be solved: cleaning of the critically colonized or biofilm loaded wound surface and turning the persisting chronic inflammation into an acute one to initiate the healing process. In recent time, tissue tolerable plasma (TTP) was distinguished to enhance wound healing [3]. We were able to show in the modified HET-CAM that plasma application can induce aseptic inflammations, which are suitable to modulate chronic inflammations [4, 5]. The antimicrobial effects of TPP are well described [6]. Still, the disadvantage of TPP is that the antimicrobial effect is immediate only and does not last long enough to prevent bacterial recolonization. We therefore combined plasma treatment with the antimicrobial substance polihexanide [7] *in vitro* and *in vivo* to add a sustained effect. Polihexanide is a first line substance for treatment of chronic wound due to its broad antimicrobial spectrum, good tissue tolerability and its ability to bind to the organic matrix. Recently, a stimulating effect of polihexanide was demonstrated [8]. We used the Reconstructed Epidermis (RE) to assess cytotoxicity effects and their reversibility (MTT – method) of combined TPP - polihexanide treatment on NHEK (normal human epidermal keratinocytes) - cells. The integrity of the RE was determined by means of the Trans-Epithelial Electrical Resistance (TEER). In the modified HET-CAM, we assessed if the combined treatment led to pronounced irritation or inflammation. The positive results from the *in vitro* assays encouraged us to test the combined treatment in two dogs. Both dogs suffered of chronic wounds for several months, conventional therapies failed. The treatment was well tolerated by both animals. In one case, the treatment led to a complete healing after 11 weeks, which was particularly remarkable because the treatment with TPP or polihexanide alone did not led to healing. This supports the presumption, that TPP and polihexanide have synergistic effects in promoting healing of chronic wounds. In the second case, the treatment was complicated by constant licking and irritation of the wound by the dog, but the healing progressed after applying a ruff and the wound has improved since then. To conclude, the applied methods (modified HET-CAM for irritation - and inflammation potential; RE for the assessment of the cytotoxicity and integrity) are suitable for screening plasma sources and parameters for medical applications alone and in combination with polihexanide. The combined TPP-polihexanide treatment is a promising option for the treatment of chronic wounds.



Fig. 1: Plasma treatment of the wound.



Fig. 2: State of the wound, 11 weeks after combined plasma-polihexanide therapy.

## References

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