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Title: An *in vitro* chronic wound model- with *in vivo* aspects

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Aim: Healing of chronic wounds is a complicated process. Bacterial biofilms play an important role in nonhealing wounds. Although a few *in vitro* and *in vivo* chronic wound models have been published, they differ in several critical areas, e.g. surface of bacteria attachment, microbial environment, geometry, temperature, and the interaction between bacteria and host. However, realistic *in vitro* chronic wound models are indispensable for initial evaluation of wound care products and understanding the microbial response towards treatment.

The aim of this study was to develop an *in vitro* chronic wound model, which mimics *in vivo* conditions.

Method: A semi-solid model was developed including an air-liquid surface interface, and a well-defined dermis layer and an underlying subcutaneous layer with high fat contents. Polymicrobial biofilms were established consisting of *Pseudomonas aeruginosa* and *Staphylococcus aureus* growing submerged in the layers. Different compositions of the layers, size of inoculum, depth of biofilm and temperature were tested.

Results/Discussion: To mimic *in vivo* compositions, components of dermis and hypodermis layers were separately optimized including e.g. serum, blood and proteins. The appropriate size of the bacterial inoculum, depth of biofilm, and temperature were established to simulate *in vivo* conditions as much as possible. A new stable and reproducible model was developed where nutrients mainly originate from blood, serum and proteins in a semi-solid agar medium, while oxygen is supplied from the top of the biofilm at the air-liquid surface interface.

Conclusion: This is a promising model to investigate the effect of various antimicrobial wound care products.

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