(117)

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Chronic Wounds in Diabetes: Molecular profiling of bacterial community and biofilm architecture

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Objectives

Chronic wounds arebiofilm infections and role of these biofilms are poorly understood. Wound biofilms consist of a diverse bacterial community where majority is uncultivable. The study investigated thepresence of structured microbial assemblages in chronic wounds was demonstrated using Scanning Electron Microscopy (SEM). Further profiling of bacteria was done by using Polymerase Chain Reaction-Denaturing Gradient Gel Electrophoresis (PCR-DGGE).

Methods

Two tissue debridement samples each were collected from fifteenchronic wound patients with diabetes. One specimen was used for SEM and Grams' stain to visualize biofilm architecture of chronic wounds. DNA was extracted and subjected to PCR which targetedthe V2-V3 region of the eubacterial 16S rRNA gene.The resulting PCR products were subjected to DGGE using30%-55% denaturing gradient. After electrophoresis the gels were stained and photographed by UV illumination. Banding patterns were compared with a reference panel which was run on DGGE gel, parallel to the samples to be tested.

Results

SEM revealed presence of bacterial biofilm communities.Biofilm-specific morphology, surface attachment, extracellular matrix and presence of individual cells and their spatial location was observed.Thisclearly provided the evidence for the involvement of biofilms in chronic wounds. In contrast Grams' staining revealed only putative microbial cells.

Based on thepresumptive identification using DGGE, every sample had more than four different eubacterial species. S.aureus was predominant followed by P.aeruginosa.

Conclusion

DGGE was found to be an effective tool in presumptive identification of aerobic and anaerobic wound pathogens. SEM images revealed a complex wound biofilm architecture.