

## OP 061

### Use of a simple gram stain method bench marked with scanning electron microscopy to identify biofilm involvement in chronic wound infections

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#### Introduction & Objectives:

Role of microbial biofilms in chronic wounds is poorly understood. Wound biofilms consist of a diverse bacterial community. The current study aimed to evaluate the use of a simple Gram stain method bench marked with scanning electron microscopy to identify biofilm involvement in chronic wound infections.

#### Methods:

Two tissue debridement specimens each were collected from fifteen patients with chronic wounds due to diabetes. One specimen was fixed in 10% formalin, histologically sectioned and Gram stained to visualize the micro-colonies by light microscopy. Safranin was used to stain the Exopolymer matrix which is the characteristic feature of a biofilm. The other specimen was fixed in 2.5% glutaraldehyde, dehydrated in a series of alcohol concentrations and subjected to gold coating and examined under scanning electron microscope to visualize biofilm architecture of chronic wounds.

#### Results:

Gram staining and safranin staining revealed the presence of bacterial micro-colonies attached to tissues which are indicative of the biofilm phenotype. However, scanning electron microscopy revealed biofilm-specific morphology with a high level of resolution. The surface attached bacterial species were embedded in an exopolymer matrix which is secreted by microbes. The evidence of biofilm involvement could be found in all tissues examined.

#### Conclusion:

Gram staining and safranin staining methods can efficiently detect micro-colonies associated with the biofilm phenotype by light microscopy. They may therefore be used for the identification of biofilms. This needs to be further validated with more number of samples.