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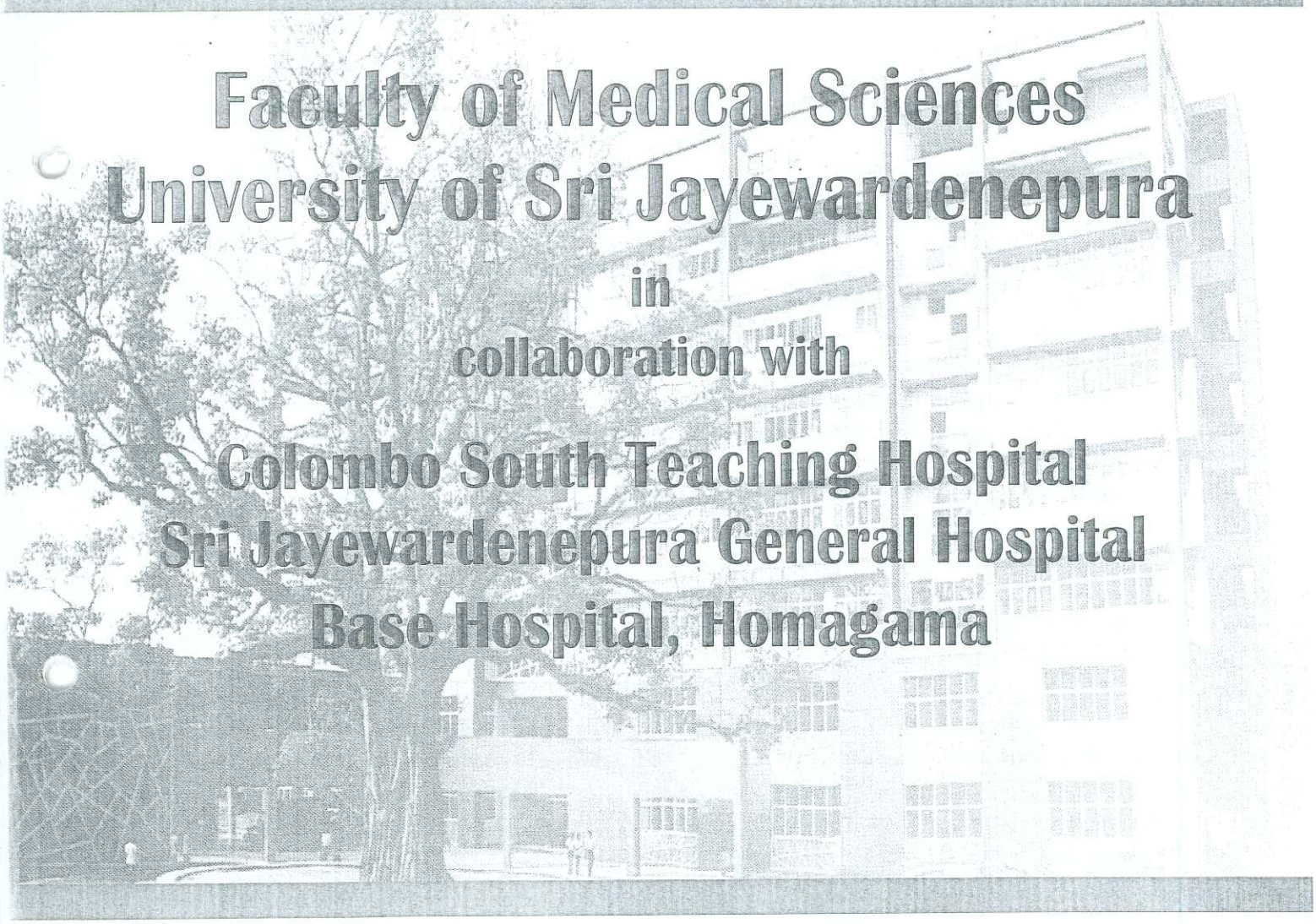

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OP 1

Efficacy of three visualization techniques: fluo rescent in situ hybridization, gram staining and scanning electron microscopy in identifying biofilm involvement of infected chronic wounds

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Background: Biofilms are one of the main contributory factors for diabetic wounds and 78% of chronic wounds contain biofilm infections. It is important to identify the biofilm involvement for effective wound management. However, there is no universally recognized method for the identification of biofilm infections.

Objectives: The study aimed to evaluate the efficacy of three visualization methods including fluorescent *in-situ* hybridization (FISH), Gram stain and scanning electron microscopy (SEM) in order to determine the involvement of biofilms in infected chronic wounds.

Methods: Twenty wound tissue debridement specimens were collected from the patients with chronic diabetic wounds attending routine surgical debridement at Colombo South Teaching Hospital in Sri Lanka. Each specimen was divided in to two. One piece of tissues was fixed in 2.5% gl utaraldehyde and used for SEM. The other piece of tissue was used for histology and Gram staining. Tissue was fixed in 10% formal saline, histologically processed, and embedded in paraffin. Serial 3μm sections were cut and subjected to Gram stain and FISH. Stained tissues were evaluated using light and fluorescent microscopy.

Results: Microbial aggregates were detected in 20/20 specimens by all three microscopic methods. SEM was more effective at highlighting the presence of exopolymer matrix and the surface attached, dense microbial cells in *in vivo* biofilms. Gram stain revealed densely aggregated micro colonies, embedded in the tissue. The use of an eubacterium specific fluorescent *in situ* hybridization probe indicated the presence of bacterial aggregates inside the exopolymer matrix, indicating putative biofilm phenotype in chronic diabetic wound tissue. Further, bacterial infiltration into internal portions of the tissues was apparent.

Conclusions: Microbial aggregates and putative biofilm matrix in wound debridement specimens can be visualized using all three methods. However, SEM was found to be the most discriminating method for the visualization of three dimensional structure of *in vivo* biofilms in fixed wound tissue specimens.

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