Detection of Bacterial Colonization and Endotoxin Content on Surgical Material and Tissue Allografts via a Rapid Visualization Assay

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Bacterial infection poses a serious problem in healthcare and the field of orthopedics. This challenge is especially problematic in implants and the insertion of fracture fixation devices, where infection rates can be as high as 28%.This high incidence rate of infections is magnified by the two million fracture-fixation devices that are inserted annually in the United States alone. In addition, these infections may lead to: extended hospital stays, increased cost, decreased quality of life, and the possibility of subsequent revision surgeries. Another potential complication is presented by endotoxin from gram-negative bacteria. Upon lysis of gram-negative bacteria, the lipopolysaccharide components of the cell wall are released as endotoxins, which are toxic substances that can cause harm to patients. Despite the significant problem with infections, current diagnostics like gram-staining, culturing, and PCR suffer from reliability, cost, length of time to diagnose, and efficiency.

This study evaluates a rapid visualization assay which employs fluorescent-conjugated antibodies and Confocal Laser Scanning Microscopy to detect the presence of bacteria and endotoxin on synovial fluid, tissues, surgical explants, and allografts in 30 minutes.

In an IRB-approved study, samples were collected by six orthopedic surgeons at Rhode Island Hospital, and were stored in 10% neutral buffered formalin. Synovial fluid samples were fixed on slides, while tissue samples and explants were analyzed in falcon tubes. All samples were stained with anti-LTA (lipoteichoic acid) antibodies conjugated to FITC488, labelling gram-positive bacterial cells green, and anti-LPS (lipopolysaccharide) antibodies conjugated to Dylight594, labelling gram-negative bacterial cells red. Images were obtained via Confocal Laser Scanning Microscopy, analyzed using ImageJ software, and compared to gram-staining images and the surgeon's clinical impression of the infection. This rapid visualization assay has the potential to be an effective diagnostic tool in the clinical setting due to its accuracy, low-cost, and ability to quickly identify the presence of bacteria.

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