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Detection, Isolation, and Raman Spectroscopic Characterization of Acute and Chronic Staphylococcus aureus Infection in an Endothelial Cell Culture Model

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Abstract : Staphylococcus aureus is a facultative intracellular pathogen, which by entering host cells may evade immunologic host response as well as antimicrobial treatment. In that way, S. aureus can cause persistent intracellular infections which are difficult to treat. Depending on the strain, S. aureus may persist at different intracellular locations like the phagolysosome. The first barrier invading pathogens from the blood stream that they have to cross are the endothelial cells lining the inner surface of blood and lymphatic vessels. Upon proceeding from an acute to a chronic infection, intracellular pathogens undergo certain biochemical and structural changes including a deceleration of metabolic processes to adopt for long-term intracellular survival and the development of a special phenotype designated as small colony variant. In this study, the endothelial cell line Ea.hy 926 was used as a model for acute and chronic S. aureus infection. To this end, Ea.hy 926 cells were cultured on QIAscout™ Microraft Arrays, a special graded cell culture substrate that contains around 12,000 microrafts of 200 µm edge length. After attachment to the substrate, the endothelial cells were infected with GFP-expressing S. aureus for 3 weeks. The acute infection and the development of persistent bacteria was followed by confocal laser scanning microscopy, scanning the whole Microraft Array for the presence and for detailed determination of the intracellular location of fluorescent intracellular bacteria every second day. After three weeks of infection representative microrafts containing infected cells, cells with protruded infections and cells that did never show any infection were isolated and fixed for Raman micro-spectroscopic investigation. For comparison, also microrafts with acute infection were isolated. The acquired Raman spectra are correlated with the fluorescence microscopic images to give hints about a) the molecular alterations in endothelial cells during acute and chronic infection compared to non-infected cells, and b) metabolic and structural changes within the pathogen when entering a mode of persistence within host cells. We thank Dr. Ruth Kläver from QIAGEN GmbH for her support regarding QIAscout technology. Financial support by the BMBF via the CSCC (FKZ 01EO1502) and from the DFG via the Jena Biophotonic and Imaging Laboratory (JBIL, FKZ PO 633/29-1, BA 1601/10-1) is highly acknowledged.

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