Investigation on host response of human monocytes by Raman spectroscopy

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As monocytes are an essential part of the immune response they hold a potential to serve as a diagnostic tool. In the course of cellular signal transduction during stress response caused by e.g. pathogens cellular membrane lipids are rearranged. The specific formation of lipid raft domains may provide biomarkers for diagnosis in sepsis associated bacteremia. Raman spectroscopy as a fast, flexible and specific technique shall be applied to investigate the monocytic host response. To perform Raman spectroscopy with monocytic cells there is a need of a gentle spatial fixation system. We want to identify and depict raft domains and reveal differences between resting and activated cells.

We established an immobilisation system with calcium alginate enabling us to perform Raman maps with both living and chemically fixed cells in the growth medium. Raman maps were recorded at 785 nm using a water immersion objective. As the single spectrum acquisition time was 1 s and the spatial step size 0.5 µm the total mapping time for a whole cell amounted to 30 to 45 min. To visualise changes in the cell upon activation hierarchichal cluster analysis was adopted as an unsupervised statistical method. The two prototypic effectors bacterial endotoxin lipopolysaccharide (LPS) and the proinflammatory mediator tumor necrosis factor α (TNFα) resulted in membrane rearrangement. Upon activation the Raman spectra of the cells considerably changed compared to the resting ones. With the help of hierarchical cluster analysis lipid raft domains were identified and visualized.