

Raman Spectroscopic Investigation of Candida albicans' Response to Neutrophils

Anuradha Ramoji (1,2), Nilesh B Kathiriya (1), Kerstin Hünninger (3), Hanna, Windecker (3), Claus Kroegel (4), Michael Bauer (1), Oliver Kurzai (3), Jürgen Popp (1,2,5), Ute Neugebauer (1,2)

(1) Center for Sepsis Control and Care, Jena University Hospital, Germany

(2) Institute of Photonic Technology, Jena, Germany

(3) Septomics Research Center, Friedrich Schiller University and Leibniz Institute for Natural Product Research and Infection Biology - Hans Knoell Institute, Jena, Germany.

(4) Internal Medicine I, Pneumology, Jena University Hospital, Germany

(5) Institute of Physical Chemistry and Abbe Center of Photonics, Friedrich Schiller University Jena, Germany

Candida albicans is a commensal fungus that can reside in the human gut and mouth without causing infections. *C. albicans* is known for its polymorphism and can grow both as yeast and hyphae. The hyphae form is invasive and especially immune compromised patients are at high risk of developing a fungal infection called candidiasis. At the onset of the infection, the fungi are first confronted with neutrophils, the first guard of the innate immune system that is responsible for eliminating the pathogens. *C. albicans* has developed some evasive mechanisms to escape the phagocytosis which are not yet fully understood.

Raman spectroscopy was shown to be a powerful method to identify different bacterial species and to differentiate between leukocyte subpopulation. In this study, Raman spectroscopy has been employed to differentiate between the *Candida albicans* confronted by the neutrophils and the non-confronted ones, based on the biochemical changes adapted by the fungi which are captured in the respective Raman spectra of the fungi.

Candida albicans SC5314 were cultivated overnight in a yeast extract peptone dextrose medium at pH 4 and 30°C. These conditions inhibited the hyphae formation. Microscopic observation confirmed the morphogenetic state of the *C. albicans* as yeast form. Polymorphonuclear neutrophils (PMNs) were isolated from the peripheral blood of healthy volunteers using density gradient method. *C. albicans* were incubated with PMNs for 1h. Control experiments were carried out under similar conditions without PMNs. Raman maps of chemically fixed *C. albicans* which were phagocytosed by the PMNs, those which escaped phagocytosis and the control group, were measured using 785 nm excitation. All the Raman measurements of the extracellular *C. albicans* were carried out on the yeast and/or budding yeast (pseudohyphae) form of the *Candida albicans*. For data analysis chemometric methods such as Nfinder algorithm, principal component analysis (PCA) and linear discriminant analysis (LDA) have been employed. A statistical classification model was built using PCA-LDA method to differentiate between the PMN confronted *C. albicans* and the non-confronted *C. albicans*. With the classification model it was possible to differentiate between *C. albicans* escaped phagocytosis and the control group. The tentative spectral analysis reveals major spectral differences arising from polysaccharides and lipids. Further investigations are being carried out to examine the difference between the phagocytosed *C. albicans* and the non-phagocytosed, escaped fungi.

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