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Raman trapping microscopy for label-free and fast cell analysis to monitor quality of cells and tissues in regenerative medicine

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Introduction:
Raman microscopy is an emerging tool in biomedicine. It provides label-free and noninvasive analysis of biological samples. Due to its high biochemical specificity, Raman spectroscopy can be used to acquire spectral fingerprints that allow characterizing cell types and states. Confocal Raman microscopy enables measurements of individual cells in 2D-cell cultures and within 3D-tissues. The integration of simultaneous laser trapping features allows reliable Raman spectra acquisition even of motile bacteria or cells within solution. This combined Raman-trapping microscopy (RTM) offers new opportunities in selective biochemical analysis and cell sorting.

Material and Method: Raman scattering is a phenomenon that occurs when incident photons of light hit a biomolecule and integrate within its chemical bonding. Molecular vibrations are induced and the photons are emitted with different energy as they become in-elastically scattered. These scattered photons are carrying the specific chemical information of the targeted biomolecule. Each molecular vibration contributes to a spectral sum of the entire cell that is consequently as characteristic as a fingerprint. BioRam® combines two Nobel prize awarded complex physical technologies – Raman spectroscopy and Optical Trapping to an easy to handle Raman-Trapping-Microscope (RTM) system. The RTM system is based on an inverted digital microscope with a large sample platform that can hold entire incubators up to 5 kg weight. The Raman-module as well as the ultra-sensitive spectrograph are optimized for cellcompatible 785 nm Raman excitation wavelength. The Raman laser is focused through the microscope objective to a focal point of about 1 µm3. This unique laser focusing enables simultaneous laser trapping of specimen in solution, held within the laser focus during Raman measurement.

Results: A droplet of bacterial solution was pipetted into the µ-channel of a channel slide with borosilicat bottom (Ibidi). Laser focus was set about 20 µm above glass bottom. Spectral pattern of bacteria species were automatically acquired using a preselected pattern with distance of about 5µm between each measure. Spectra of 20 individual bacteria provided a clear specific pattern. Raman spectra of stem cells cultured under normal conditions were compared with cells treated under different conditions. Clear differences within the spectra depicted the influence of culture conditions. Primary human tracheobronchial epithelial cells where characterized using RTM. Discrimination from tumor cells warrants construction of healthy tissue models.
Furthermore RTM was used to monitor the quality of skin cells and autologous skin grafts and to proof condition as well as functionality of blood products. Here spectra of about 100 fibroblasts, keratinocytes or melanocytes where measured yielding clear spectral differences within cell types. Raman spectra within the depth (200µm) of the final graft depicted quality of the cells (i.e. identify amount of decayed cells). In case of blood products spectra of erythrocytes and thrombocytes were measured at different time points to monitor spectral changes with time.

Discussion: The fast and sample preserving method provides new insight into the behavior of cells within liquid, 2D-cell cultures and 3D-tissue. Consequently, Raman microscopy is a promising tool that has great potential to become standard in quality control of advanced therapeutic medicinal products.