Differentiation of individual human mesenchymal stem cells probed by FTIR microscopic imaging

Christoph Krafft[1], Matthias Schieker[2]

[1]Bioanalytical Chemistry, Dresden University of Technology, D-01062 Dresden, Germany
[2]Experimental Surgery and Regenerative Medicine, Department of Surgery, Ludwigs-Maximilian University Munich, Nussbaumstr. 20, D-80336 München, Germany

A major factor of uncertainty in cell biology and cell developmental studies is that the state of cell differentiation is not exactly known. New methods which can be applied non-manipulatively and non-destructively would constitute a significant progress over traditional label-based techniques as they are simpler and faster, and even mixed cell populations with dynamic growth patterns could be monitored continuously and under *in vivo* conditions. An important application is to control the proliferation and differentiation of various cell types originating from stem cells which differ in function, structure and composition. Differentiation of stem cells can be stimulated in vitro by external reagents or change of growth media. This property makes them an attractive candidate for innovative medical applications such as tissue engineering of bone using human mesenchymal stem cells (hMSCs). Substitutes loaded with autogenous and allogenous stem cells and subsequently implanted in a bone defect which could be caused by trauma, tumor or infection show regularly new bone formation of good biomechanical stability. But the detection of implanted cells in rodents is a frequently arising problem after xenotransplantation, so that the exact function and participation of implanted cells on new bone formation remains unclear.

Infrared (IR) spectroscopy offers the possibility to overcome some of the restrictions and to complement standard methods as a novel analytical tool in cell studies. The principle is that each cell type and each cell state are characterized by specific molecular properties which are represented by sensitive IR spectroscopic fingerprints. Knowing the correlation between the cell property and its IR spectrum allows the IR-based classification. This proof-of-concept study utilized the advantages of FTIR microscopic imaging for the first time to collect 262144 IR spectra from several hundred individual hMSCs at diffraction limited lateral resolution. A classification model based on linear discriminant analysis was developed which distinguished four differently differentiated hMSC populations in four independent preparations on the single cell level. Without osteogenic stimulation two cell types dominated showing low or high levels of glycogen accumulation at the cell periphery. After osteogenic stimulation the protein composition in cells changed and some cells started expressing calcium phosphate salts such as octacalciumphosphate, a precursor of the bone constituent hydroxyapatite. Few cells were identified in the FTIR images which remained in their non-stimulated state.

Methods: After 7 days of cultivation in osteogenic or non-osteogenic medium cells were trypsinized and allowed for adherence over night on calcium fluoride slides. Following, cells were fixed in 100% methanol (15 min), washed in PBS, dried and stored at -20°C. Mosaics of 16 FTIR microscopic images were sequentially recorded in a 4×4 pattern which covered areas of 1064×1064 μm^2 using the Hyperion system equipped with a FPA detector of 64×64 pixels (Bruker Optik, Germany). Data analysis was performed by an in-house developed graphics user interface operating under a Matlab platform (The Mathworks, USA).

References

C. Krafft, R. Salzer, S. Seitz, C. Ern, M. Schieker. Analyst (2007) 132: 647-653.