Label-free and non-destructive: A novel technology to analyze stem cell behavior

R. Gangnus[1], V. Charwat[2], C. Kasper[2], C. Klopsch[3], K. Schütze[1]

[1]CellTool GmbH, Bernried, Germany, happycells@celltool.de;
[2] Department for Biotechnology, University of Natural Resources and Life Sciences BOKU, Vienna
[3] Department of Cardiac Surgery, Reference- and Translation Centre for Cardiac Stem Cell Therapy (RTC), University of Rostock

Introduction

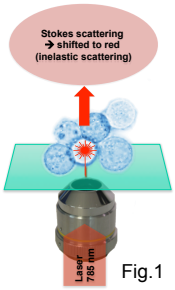

Fig.1

Raman spectroscopy is a molecular spectroscopic technique, based on the detection of light that has been inelastical scattered by a sample (“Raman effect”). It is based on focused laser light shone into cells to excite molecular vibrations (Fig.1). The shift in frequency of the emitted light is detected by a spectrograph.

Raman scattering results in well-resolved peaks, uniquely **associated with the biochemical properties** of the samples (Fig. 2).

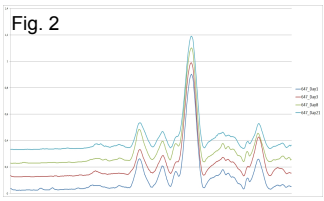
Fig. 2

This information can be used to **identify and characterize** cell types, internal cellular states or the cellular response onto external influences such as drugs or environment.

Our aim was to demonstrate if it is possible to monitor stem cell behavior in a fast, non-destructive and label-free way. In this work we present Raman spectroscopy as a new tool for gentle and non-invasive survey of stem cell growth and differentiation on two independent examples.

Methods

In the first application Raman spectroscopy was used to investigate stem cells isolated from adipose tissue exposed to varying **oxygen conditions**. Therefore, cells were cultivated either under standard conditions (21% O2) or under hypoxic conditions (5% O2). Additionally, cells were subjected to hypoxic conditions for short time periods of 2 and 4 days to observe dynamic changes.

In the second application we focused on the influence of **erythropoietin (EPO)** in the differentiation of mesenchymal stem cells.

In both cases analysis of Raman spectra was used to monitor the behavior and change of the cells.

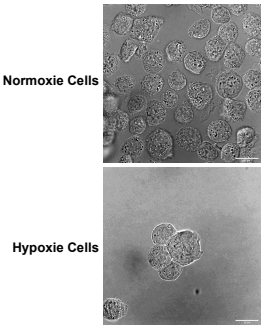

Fig. 3 – Stem cells isolated from adipose tissue exposed to varying oxygen conditions

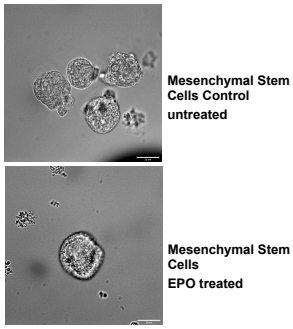

Fig. 4 – Differentiation of mesenchymal stem cells influenced by EPO

Results

In the first application we could clearly identify hypoxia cells (red lines) showing different profiles compared to control cells in standard conditions (blue lines). The **RNA expression** level was found as differentiator.

Additionally, Raman revealed a trend from standard conditions towards hypoxia over time (see Fig.4 – negative values for standard conditions move over time to positive values in the PCA lineplot). This was shown with cells first cultured for 4 days under standard conditions and then exposed for 2 days (aubergine) and 4 days (pink) to hypoxic conditions, respectively. The majority of cells of the 4 days test are clearly located opposite (positive lines) to the normal treated cells (negative lines). However, cells that have been grown from the beginning under hypoxic condition seem partly to be adapted to this conditions and behave like “normal” cells (negative lines).

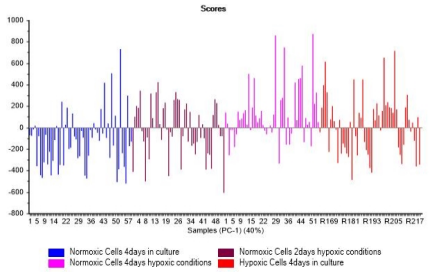

Fig.4 PCA Analysis – LinePlot shows separation of “Normoxia cells” (negative values) from Hypoxia cells (positive values).

Results of the second application support the hypothesis that EPO treatment induces MSCs to differentiate towards fibroblasts. Fig.5 shows two distinct clusters of arterial fibroblasts (blue) and untreated MSCs (green & bright green). The majority of EPO treated MCSs (red) cluster within the region of untreated MSCs, but there is a group of cells (about 35%) behaving like arterial fibroblasts (see red circles).

This behavior was also confirmed with gene profiling experiments.

The investigated two different media DMEM (green) and MSCGM (bright green) did not show an influence on Raman spectra.

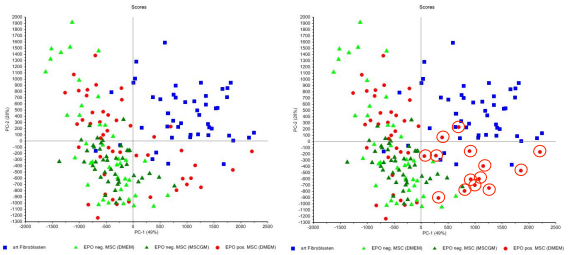

Fig.5 PCA Analysis – ScorePlot shows a clear separation of arterial fibroblasts (blue) to untreated MSCs (green: in DMEM medium; bright green in MSCGM medium). EPO treated MCSs (red) cluster within the region of untreated MSCs, but some cells (about 1/3) are assembled within the arterial fibroblasts region (red circles).

Conclusion

These two examples show that Raman spectroscopy is a fast and non-invasive technology to identify, characterize and monitor stem cell behavior. The method could even be applied *in-line* during ongoing cell culture.

References:
1) Z. Movasaghia et al.; Raman Spectroscopy of Biological Tissues, Applied Spectroscopy Reviews 42,5: 493-541, 2007

This project has received funding from the European Union’s Seventh Programme for research, technological development and demonstration under grant agreement No 279288.

happycells@celltool.de
www.celltool.de