Towards the identification of hair follicle mesenchymal stem cells by FPA-FTIR

K. Lau[1,2], B. R. Wood[3], J. Kloepper[4], K. Bambery[3], R. Paus[4] and V. Deckert[1,2]

1.Institute of Physical Chemistry, FSU-Jena, Lessingstr., 10, 07743 Jena, Germany
2.Institute for Photonic Technology, Albert-Eintein-Str 9, 07745 Jena, Germany
3.Centre of Biospectroscopy, School of Chemistry, Monash University, Australia
4.Department of Dermatology, UK-SH Lübeck, 23538 Lübeck, Germany

The hair follicles in the skin present a rich source of stem cells important in regenerative medicine. In particular, the mesenchymal stem cells (MSC) located in the mesenchyme – dermal papilla(DP) and connective tissue sheath(CTS)- of the hair follicle, have been shown to be multipotent[1],[2] and participate in the dermal regeneration during cutaneous wound healing[3]. So far, there are no definitive markers for the human hair follicle MSC apart from their nestin expression, hindering the progress of isolating, characterising and exploiting them in regenerative medicine. In order to develop a label-free and non-destructive method to identify the human hair follicle MSC, Focal Plane Array (FPA) Fourier Transform Infrared (FTIR) microspectroscopy was employed. Following our initial success of using FTIR to discern individual tissue layers based purely on their spectral features, a map scan was performed over the hair follicular mesenchyme to focus on the MSC niche.

A 5 µm cryosection of human scalp skin, containing a cross section of an anagen VI hair follicle, was mounted on a Kevley Low 'e' slide^{TM}. The section was fixed by 4% paraformaldehyde at room temperature, washed three times to remove the optimal cutting temperature embedding compound thus preventing it from contaminating the sample. The measurement was carried out in reflection mode using a Bruker Optics Hyperion 3000 FPA FTIR instrument, equipped with a 64x64 FPA detector. The setting applied permitted approximate single cell spatial resolution. The data were processed using Cytospec software, by first removing spectra from regions outside the follicle and performing an unsupervised hierarchical clustering analysis (UHCA) over the 1800-1000 cm^{-1} spectral region selecting a low number of clusters. From the clustering position, clusters formed by spectra from the mesenchyme can clearly be distinguished. By selecting the mesenchyme clusters as the region of interest (ROI) and further performing UHCA on the 1st derivatives of the spectra of the ROI, over 1800-1000 cm^{-1}, the mesenchyme was selectively divided into clusters based on the similarity of the spectra. The clusters clearly display variation in their protein, lipid and nucleic acid compositions.

By pairing the information of the biomolecular composition gathered from the 1800-1000 cm^{-1} region and information of the fatty acids composition from the 3100-2800 cm^{-1} region, we have narrowed down the approximate position in the hair follicle where MSC are likely to be found. This will be verified by immunostaining of nestin, an interfilament protein known to be expressed by hair follicle MSC, in the near future. This study has utilised a novel method to selectively cluster spectra from a region of interest.

References:

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