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Measuring concentration depth profiles and stratum corneum thickness in vivo

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Introduction

Confocal Raman spectroscopy (CRS) is an upcoming spectroscopic technique. It belongs to the field of vibrational spectroscopy and provides insight into the vibrational states of a molecule. The fact that it is non-invasive offers several advantages compared to other methods; an *in vivo* application is possible. In the area of skin research, this optical method allows the depth-resolved measurement into the skin, non-invasively and non-destructively.

The aim of the present study was to characterise the skin composition of the volar forearm using CRS. Skin components of interest were NMF and urea. These were selected according to their influence on the skin state and barrier function. Additionally, SC thickness was calculated from water concentration profiles.

Experimental methods

Study design

The study was conducted with approval from the Ethics Committee of the Medical University of Vienna. Fifteen randomly chosen Caucasian volunteers of both sexes, aged between 23 and 57 years, participated in the study. CRS experiments were carried out twice within 2 weeks. During data evaluation, noticeable differences in CRS profiles were found that seemed to be linked to age. To assess this assumption more closely, two extreme age groups were defined: a young group consisting of two participants <25 years of age and an elderly group consisting of two participants >50 years of age.

Confocal Raman spectroscopy (CRS)

In vivo CRS experiments were carried out using a confocal Raman microspectrometer (gen2 Skin Composition Analyzer, River Diagnostics, Rotterdam, The Netherlands) with two incorporated lasers (671 nm and 785 nm). Fingerprint spectra were recorded from 0 up to a depth of 32 µm in 4 µm increments. Spectra in the high wavenumber region were obtained in 2 µm steps up to a depth of 40 µm. All spectra were measured on the volar forearm. At least five fingerprint profiles



and three high wavenumber profiles were collected and averaged for each volunteer. All spectra collected were analysed using SkinTools® software version 2.0, developed by River Diagnostics. The concentration of the skin components NMF, urea, ceramide and cholesterol was calculated as described by Caspers et al. [1]. Water profiles were generated by calculating the water content from the water to protein ratio. SC thickness was determined through water concentration profiles [2].

Results

The formation of NMF is reflected in the concentration profiles generated within the present study. NMF is formed by degradation of the protein filaggrin, which itself arises out of profilaggrin conversion. The transformation of filaggrin to NMF starts at the lowermost part of the SC and proceeds through the upward layers. In deeper skin areas, filaggrin does not persist. Our findings showed a substantially increased NMF concentration at the skin surface compared to deeper layers.

Age-dependent variations of the skin were examined. Thereby, an elevated amount of NMF in aged skin was found.

In contrast to NMF, the urea concentration profiles did not show a consistent progression. For one group of the volunteers, within 32 µm depth almost no changes in the urea concentration were found. The other group showed a considerable higher urea concentration at the skin surface, followed by a rapid drop. The comparison of the two did not reveal any significant age-related differences.

CRS-derived SC thicknesses varied between 12 and 26 µm. SC thickness was expected to increase with age; indeed, notable differences between the age-group of under-25-years-old and the participants over 50 years of age were found. The apparent SC thickening with age may be caused by the also observed decline in the water concentration.

In conclusion, depth profiles showed a comparable curve progression for the tested individuals and age-related differences in skin composition were found. CRS serves as useful tool to investigate skin properties fast and without the need for pre-treatment.

Acknowledgements

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References

- [1] P.J. Caspers, et al. *In vivo confocal Raman microspectroscopy of the skin: noninvasive determination of molecular concentration profiles.* J. Invest. Dermatol. 116 (2001) 434–42.
- [2] M. Egawa, et al. *In vivo estimation of stratum corneum thickness from water concentration profiles obtained with Raman spectroscopy.* Acta Derm. Venereol. 87 (2007) 4-8.

