Quantification of Natural Moisturising Factors at the skin surface using a portable infrared spectrometer device: a pilot calibration model

P048

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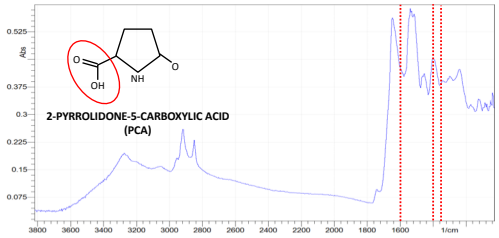
AIM: To combine Infrared Spectroscopy with chemometric analysis to model surface Natural Moisturising Factor (NMF) levels *in vivo*

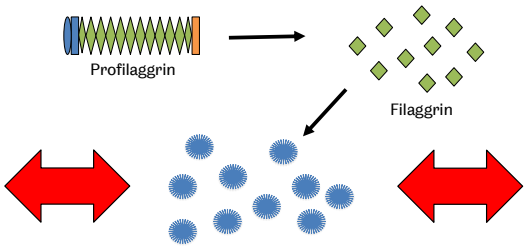
- **NMF is a biomarker of *FLG* status[1] and skin dryness[2]. *FLG*-related Atopic Dermatitis (AD) is associated with more severe / persistent disease[3]**

CURRENT METHODS OF NMF QUANTIFICATION

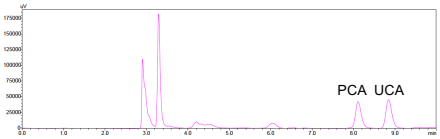
1) *In vivo* Fourier Transform Infrared Spectroscopy (FTIR)



FTIR spectra of skin showing NMF carboxylate signal (red) at 1600,[4] 1410[5] and 1340cm^{-1} wavenumbers



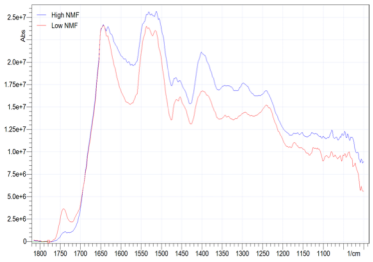
2) *Ex vivo* Laboratory analysis of tape strips



- Isocratic elution of 2-Pyrrolidone-5-Carboxylic acid (PCA) and Urocanic acid (UCA) in potassium phosphate pH2.5 by HPLC monitored at 210 and 270nm[6]
- Free amino acids (FAA) quantified by o-phtaldialdehyde derivitisation[7]

PARTIAL LEAST SQUARES REGRESSION MODELLING OF *in vivo* FTIR vs *ex vivo* TAPE STRIPPED NMF LEVELS

- 4300 FTIR device equipped with 2-bounce, 3-pass accessory and cooled MCT detector (Agilent, USA)
- Mid infrared region 1710-1185cm^{-1} modelled
- Chemometric analysis performed using MicroLab expert (Agilent, USA)

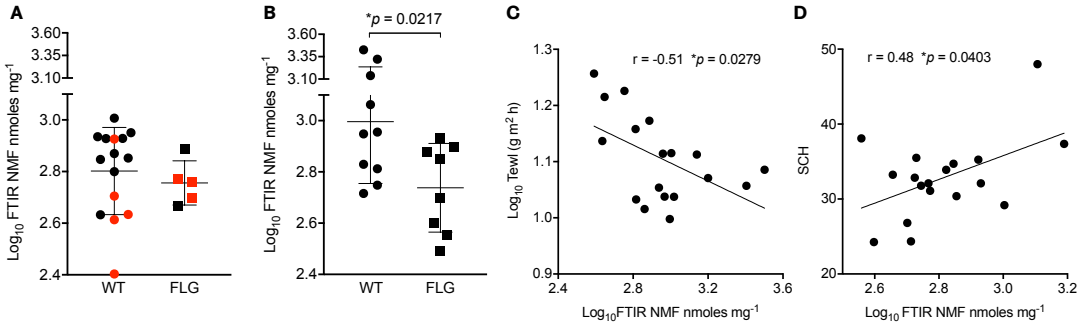


4300 FTIR device

- 10 healthy and 12 AD patients
- UK working party criteria[8] and EASI[9] used for AD diagnosis and severity
- 2x FTIR spectra collected from the forearm and antecubital fossa
- Tape strips (TS) 1-3 collected in duplicate and analysed *ex vivo* for NMF levels
- NMF (nanomoles / mg) = the sum of PCA, UCA and free amino acids (FAA) normalised to mg of SC removed (squamescanner device)[10]

Panel 1: Modelling surface NMF at the cubital fossa using IR region 1710-1185cm^{-1}. **Left panel:** Spectral variation due to high and low NMF. **Right panel:** Partial least squares regression of FTIR vs TS NMF levels

MODELLED NMF VALUES CORRELATE WITH *FLG* GENOTYPE, SKIN DRYNESS (CORNEOMETRY) AND TEWL



Panel 2: FTIR NMF modelling at the antecubital fossa surface in **(A)** AD and **(B)** Healthy subjects with and without (WT) a *FLG* mutation. Subjects with active lesions are highlighted in **red**. All participants were genotyped for the 5 most common European *FLG* mutations: R501X, 2282del4, R2447X, S3247X and 3702delG.[11] Modelled FTIR NMF levels correlated with **(C)** barrier function and **(D)** stratum corneum hydration in healthy subjects. Capacitance and TEWL were measured using a corneometer (C&K, Germany) and AquaFlux evaporimeter (Biox, UK). Log_{10} values were calculated to generate more normal distributions

CONCLUSIONS

- FTIR combined with chemometric analysis is well suited for the instantaneous *in vivo* quantification of NMF at the skin surface
- Further model validation in larger cohorts is required
- The use of a portable FTIR device makes this methodology suitable for any clinical setting
- *In vivo* quantification of NMF provides information on the inherited[1] and acquired[12] FLG deficiency, and may inform long term clinical treatment strategies in AD

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