

# Effects of Topical Moisturizer Application on Stratum Corneum Water Gradient Measured *In-Vivo* with Confocal Raman Microspectroscopy

JM Crowther<sup>1</sup>, PJ Matts<sup>1</sup>, JR Kaczvinsky<sup>2</sup>,  
P&G Beauty, Egham, UK<sup>1</sup> and Cincinnati, USA<sup>2</sup>

## INTRODUCTION

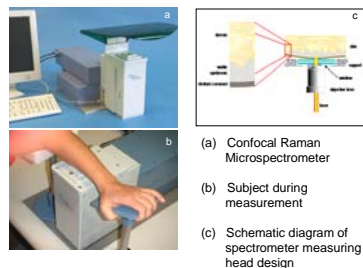
The stratum corneum (SC) water concentration gradient is fundamental to skin's role as a barrier, regulating its physical and biochemical properties.<sup>1</sup> Standard instruments which utilize changes in SC electrical properties to estimate SC water concentration provide simple, rapid measurements. However they cannot provide true interval data as a function of depth. Confocal Raman Microspectroscopy (CRM) of human subjects provides non-invasive, real-time, *in-vivo* measures of molecular concentration profiles.<sup>2,3</sup>

## METHODS

### Confocal Raman Spectroscopy

3 prototype water in silicone emulsion based moisturizers; a vehicle control (A), one with a Niacinamide containing vitamin complex with known barrier properties (B), and one with Niacinamide and Hexamidine (C), were applied twice daily for 4 weeks at a rate of 2mg/cm<sup>2</sup> according to a pre-determined randomization, to skin sites within the volar forearms of n=32 subjects with normal skin. One site was left untreated throughout the study as a control (treatment regime U). At baseline, 4 weeks treatment and after 1 week of regression (cessation of treatment), CRM measurements (8 replicates) were obtained from each site as detailed below.

A state-of-the-art confocal Raman microspectrometer equipped with a fiber-coupled laser source operating at a wavelength of 671 nm was used to obtain measurements in the high wavenumber region (~2400-4000 cm<sup>-1</sup>). An air-cooled, high-sensitivity back-illuminated, deep-depletion CCD camera captured radiation scattered inelastically from focal planes within the skin *in-vivo* (a high-precision, computer-controlled piezo-electric stage and objective allowing depth resolutions of <5 µm, with over-sampling). High-wavenumber data were analyzed to provide semi-quantitative measures of water concentration ([water] / [protein + water]) across the SC. From the water profiles generated, SC thickness and total hydration levels can be calculated.



### TEWL measures

TEWL measurements were taken from each site at each timepoint, using a commercially available apparatus. These were measured before the CRM profiles were collected.

We have used CRM to look at the chronic effects of 3 topical moisturizing treatments – one with a Niacinamide containing vitamin complex in combination with Hexamidine, one with Niacinamide alone, one without Niacinamide (Niacinamide possesses known SC barrier enhancing properties)<sup>1</sup>. CRM provides information on the distribution of water within the SC, and from this it is possible to calculate a SC thickness and a measure of total hydration. Unlike traditional measures CRM produces an actual water profile within the SC. The results of this study are reported here.

## RESULTS

### Confocal Raman Microspectroscopy

The water concentration profiles from all subjects and sites were pooled and plotted together for each timepoint of the study. At week 4 (the end of the treatment phase), the shape of the water concentration gradient for the moisturizers containing the Niacinamide/vitamin complex and Niacinamide plus Hexamidine were significantly different from the other sites (see Fig 1 below) – a progressive “stretching” of the SC water gradient. This effect remained at the 1 week regression timepoints. This reflected an increase in depth of the SC (validated by comparison with OCT measurements not shown here) and, therefore, this depth change needs to be taken into account in analysis of the water gradients. Concomitantly with measuring changes in SC thickness, the CRM profiles also calculate a total SC hydration level, based on the area under the curve.

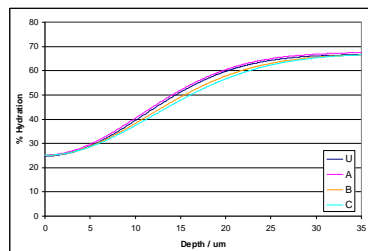


Figure 1 – CRM average water profiles per treatment after 4 weeks product usage

Previous CRM studies have also shown this effect of skin plumping for certain moisturizers.<sup>4,5</sup> Based upon this, custom algorithms were written to analyze the water concentration gradient data, taking into account SC depth changes. The data from these analyses were expressed as total SC water holding capacity and are shown in Fig 2. By week 4, the moisturizers containing Niacinamide (products B & C) had increased total SC water content significantly more (p<0.05) than both the non-Niacinamide containing vehicle control A and the untreated control U. This magnitude of the change corresponded to a 8-10% increase in SC thickness and total water content from the baseline. Both Products B and C showed this change remained after 1 week regression indicating a true effect on the underlying SC biology. Importantly though, addition of Hexamidine had the effect of prolonging this change in thickness during the regression phase.

## OBJECTIVE

- Use CRM to demonstrate an increase in total hydration level of the SC for moisturizers with a Niacinamide containing vitamin complex both in a long term usage situation and in the regression phase of treatment.
- Demonstrate additional regression phase skin hydration benefits when combining Hexamidine with Niacinamide.
- Show the potential for CRM to explain observed changes in SC barrier function.

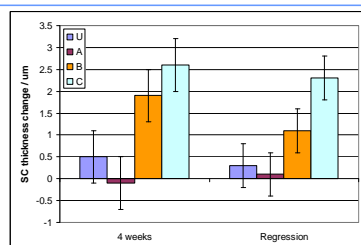


Figure 2 – CRM derived mean % change in total SC hydration vs baseline

Comparing the CRM total hydration changes with TEWL values during the study, we can see that with the TEWL measure, addition of Niacinamide provides a barrier strengthening effect after 4 weeks, Figure 3, which remains into the regression phase. This additional information can provide insights into the underlying mechanisms of moisture retention and loss within the SC layer, as well as explain the increase in barrier integrity for the Niacinamide and Niacinamide/Hexamidine containing products. For example a greater depth of SC, composed of compact corneocytes (and maintaining a smooth water gradient across it) would be expected to have increased barrier function as water passing through would move along a more tortuous path.

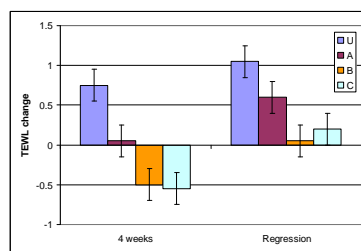


Figure 3 – mean TEWL change vs baseline

We have published previously on the wide-ranging effects of Niacinamide in human skin, notably its ability to up-regulate endogenous ceramide synthesis and expression of key structural proteins (including filaggrin and involucrin), critical to SC barrier function. It would appear, therefore, that the results reported here provide new mechanistic insight into the well-characterised augmentation of SC barrier function driven by topical Niacinamide<sup>6</sup>, and an augmentation of the longevity of this effect when used in combination with Hexamidine.

## CONCLUSIONS

- CRM has shown the significant and unique effects of a topical moisturizers with a Niacinamide containing vitamin active complex. Significant increases of approximately 8-10% in SC thickness and total hydration level, when compared with the baseline condition, were seen for the Niacinamide containing moisturizers in both the treatment and regression phases. These changes were not seen for the non-Niacinamide containing product or the untreated leg.

- Addition of Hexamidine to the Niacinamide formula resulted in an extended maintenance of this skin plumping effect over Niacinamide alone after ceasing product usage (1 week regression).

- CRM correlated well with barrier function (TEWL), demonstrating the potential for this technique in further understanding SC hydration from topical moisturizers.

## REFERENCES

1. AV Rawlings et al., J. Invest. Dermatol., 124, 2005, 1099-1110.
2. PJ Caspers et al., J. Raman Spectr., 31, 2000, 813-818.
3. PJ Caspers et al., J. Invest Dermatol., 116(3), 2001, 434-442.
4. A Sieg et al, Proc. SPIE, Vol. 6093, 2006, 60930N.
5. JM Crowther et al., Br. J. Dermatol., epub 2008, June 28<sup>th</sup>.
6. D Bissett, Cutis, 70 (6 suppl), 2002, 8-12.