32nd IFSCC Congress
London 19-22 September 2022

Demonstrating permeation of an anti-ageing peptide into the *stratum corneum* using 3D OrbiSIMS

Mark O'Mahony[1]; Stefanie Kern[2]; Mohammed Khan[2]; Mark Johnson[1]; David Scurr[2]; Mike Bell[1]

[1] No7 Beauty Company, Walgreens Boots Alliance, Nottingham, UK
[2] School of Pharmacy, University of Nottingham, Nottingham, UK

Poster ID: 455

Introduction:

Crossing through the *stratum corneum* is the rate limiting step in the permeation of skincare active ingredients into the underlying epidermis and beyond. Tracking and profiling the permeation of these ingredients through the *stratum corneum* can be challenging, particularly if the chemistry of the active ingredient is similar to the native chemistry of the skin.

Here, a state-of-the-art 3D OrbiSIMS approach has been used to demonstrate the permeation of the biologically active anti-ageing peptide Palmitoyl Tripeptide-1 (Pal-GHK) into the *stratum corneum*. Pal-GHK is widely used in the cosmetic market [1, 2] and is a key component of the active ingredient Matrixyl 3000™. This peptide is composed of a fragment found in the collagen-1 protein and is chemically similar to peptides found in nearly 3000 different proteins in the skin. Therefore it is challenging to measure permeation profiles of Pal-GHK. The 3D OrbiSIMS combines secondary ion mass spectrometry (SIMS) with the high mass-resolving power of an Orbitrap™ mass analyser, facilitating *in situ* label-free molecular analysis and the identification of organic species in complex solid samples, including biological tissues [3].

Materials & Methods:

In vivo Study

Twelve Caucasian subjects (11 female, 1 male; aged 30-55) were treated with three serum-type formulations containing <100 ppm Pal-GHK applied at 2mg/cm^2 to the volar forearms, with one area remaining untreated. Four hours post-application, 15 sequential tape strips (22mm diameter) were collected from all sites using D-squame® tape strips (CuDerm). Optical absorption of the tape strips was measured in triplicate at 850 nm to determine skin cell density. 3D OrbiSIMS was then used to determine the presence and intensity of the Pal-GHK peptide at 3 separate points on the tape strip. The molecular peak intensity was normalised against total ion content and an average of the triplicate values taken. The average molecular peak intensity was subsequently normalised against the average optical absorption of corresponding tape strips, therefore adjusting for inter-subject variability in the amount of stratum corneum captured in each tape strip.

Ex vivo Study

Full thickness human skin tissue was removed during cosmetic surgery from Caucasian female donors (aged 35-70). The explant was cut and mounted, dermal side down, in a Franz-type static diffusion cell set-up [4], with an exposed surface area of 1.1 cm^2. Infinite doses of formulations containing Pal-GHK at <100 ppm were applied to the donor chamber, with the explant exposed to the formulations for 4 hours in a water bath set to 36.5 °C. Sink conditions were maintained throughout the experiments. After 4 hours, the Franz cell was dismantled, excess formulation was removed and the explant was dehydrated under vacuum at room temperature for 24 hours before 3D OrbiSIMS analysis.

3D OrbiSIMS Analysis

3D OrbiSIMS Analysis was performed on a Hybrid SIMS instrument (IONTOF GmbH) under the following conditions; A 20 KeV Ar3000+ analysis beam with a diameter of 20µm was used as the primary ion source. Samples were analysed at ambient temperature across a 400 × 400µm area in positive polarity with sawtooth raster mode and a total crater size of 486 × 486µm. Duty cycle was set to 4.4% and cycle time to 200µs. Mass spectra were recorded at a resolution of 240,000 at m/z 200 in the mass range of 75 to 1,125 m/z. Both data acquisition and the subsequent data processing were performed using SurfaceLab 7 software (IONTOF GmbH).

Results & Discussion:

The Pal-GHK molecular ion, $C_{30}H_{55}N_6O_5$+ was used as the diagnostic marker throughout the analysis, having first been confirmed to be present and absent in the formulations and unused blank tape strips respectively.

In vivo Study

The depth profile of Pal-GHK across 15 *stratum corneum* tape strips was determined for 3 different serum-type formulations each containing <100 ppm Pal-GHK in 3 subjects. The Pal-GHK peptide ion signal was detected right through the 15 tape strips for all 3 formulations (Fig. 1). Based on the results of these 3 subjects, 5 tape strips centred on tape strip 10 (tape strips 8-12) were chosen for further analysis across 12 subjects. Pal-GHK could be clearly detected in tape strips 8-12 (Fig. 2), and while there was some variability between the formulations, there was a significant difference versus the untreated control for all samples (Student's t test, $p<0.05$). The data was further interrogated to focus on tape strip layers 10-12 of the 12 subjects (Fig. 3). A considerable amount of Pal-GHK peptide was detected in tape strips 10-12 for all 3 formulations, with over 65% of subjects having detectable peptide at tape strip 10 or beyond (Student's t test, $p<0.05$ vs untreated control). Therefore, these data shows that Pal-GHK from all 3 formulations permeates to at least 10 surface layers deep in the majority of subjects.

Ex vivo Study

A formulation containing <100 ppm Pal-GHK was applied to *ex vivo* human skin in a Franz-cell chamber. The tissue was treated for 4 hours before being analysed with the 3D OrbiSIMS. The ionising beam of the OrbiSIMS sputters the surface of the tissue, with increased sputter time corresponding to increased depth into the tissue. The data demonstrated that the Pal-GHK molecular ion was clearly detected, with the ion intensity decreasing with increasing skin depth, reaching a baseline level at the *stratum corneum* – living epidermis junction (Fig. 4). The *stratum corneum* – living epidermis junction was located to a sputter time of approx. 4000s in previous work by tracking the secondary ion markers of phospholipids (data not shown).

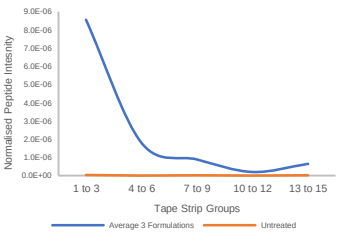

Fig. 1: *Peptide Intensity of Pal-GHK Across Entire Depth Profile. Data shown is the totalled peptide intensities for each set of three tape strips, averaged across 3 serum-type formulations, compared to the untreated control, for 3 subjects.*

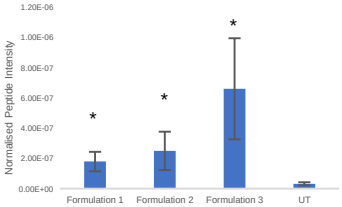

Fig. 2: *Total Peptide Intensity in Tape Strips 8-12; Data shown is average total peptide intensity in tape strips 8-12 for n of 12, +/- SEM, for three formulations and the untreated control (UT), * p<0.05 v untreated.*

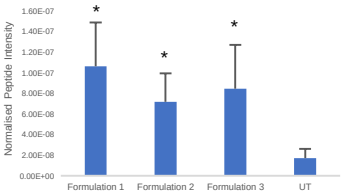

Fig. 3: *Total Peptide Intensity in Tape Strips 10-12; Data shown is average total peptide intensity in tape strips 10-12 for n of 12, +/- SEM, for three formulations and the untreated control (UT), * p<0.05 v untreated, for >65% subjects in which peptide was detected in tape strip 10 or above*

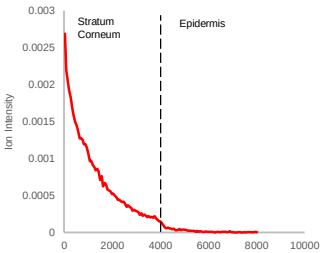

Fig. 4: *Ex vivo Permeation of Pal-GHK; the stratum corneum-epidermal junction is marked with a dashed line.*

Conclusions:

Despite the low concentration of Pal-GHK within the skin and its chemical similarity to native skin components, this novel approach clearly identified the molecular ion and facilitated assessment of the permeation of the peptide as a function of skin depth; data that has previously been difficult to accurately attain. 3D OrbiSIMS has enormous potential to provide molecular information for skin research and further understanding of active ingredient delivery. Indeed, we have recently published further research where the 3D OrbiSIMS was used to characterise the complex chemistry of the skin, particularly that of the *stratum corneum* [5].

References:

[1] Ferreira MS, et al (2020) Trending anti-aging peptides. Cosmetics 7:91.
[2] Jariwala N, et al. (2022) Matrikines as mediators of tissue remodelling. Adv Drug Del Rev 185:114240.
[3] Passarelli MK, et al (2017) The 3D OrbiSIMS – label-free metabolic imaging with subcellular lateral resolution and high mass-resolving power. Nature Methods 14:1175-1186.
[4] Franz TJ (1975) Percutaneous absorption; On the relevance of in-vitro data. J Invest Dermatol 64:190-195.
[5] Starr NJ, et al (2022) Elucidating the molecular landscape of the stratum corneum. Proc Natl Acad Sci USA 119:e2114380119

Acknowledgements:

This work was funded by Walgreens Boots Alliance