CORRELATIVE RAMAN AND MASS SPECTROSCOPIC IMAGING TO QUANTIFY DRUG DELIVERY INTO THE SKIN

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PURPOSE

- Methods to assess drug bioavailability (BA) in skin indirectly infer rate and extent of delivery.
- Confocal Raman spectroscopy can track drug penetration into viable skin beneath stratum corneum (SC).
- However, robustness of Raman data requires independent validation with correlative techniques

OBJECTIVES

To demonstrate and confirm, using independent imaging methods, that confocal Raman can quantify drug input into skin.

METHODS

- **Skin samples** (pig abdominal) were used *in vitro.*
- 6-hour application of fully or 25% saturated (170 or 42.5 mg/mL) 4-cyanophenol (CP; selected for its strong Raman signal in the infrared-transparent frequency range of the skin) in 50:50 v/v water/propylene glycol.
- Post-treatment, formulations removed, and skin surface cleaned.
- **Confocal Raman** spectra acquired with a Renishaw inVia microscope (785 nm laser).
- CP signal (C≡N vibration, 2230 cm^{-1}) normalised by corresponding amide I intensity at 1650 cm^{-1}.
- Normalised CP intensities converted to concentrations using calibration curve generated in a rehydrated lyophilised pig skin powder model.
- **Stimulated Raman Scattering (SRS)** images acquired with Leica SP8 microscope.
- CP disposition in the skin assessed 'top down' and in 'side-view' 30 µm thick cryotomed cross-sections.
- Signals acquired from C≡N and amide I, from CH_2 (skin lipids, 2850 cm^{-1}), and second harmonic generation (SHG) from collagen.
- CP signal again normalised using amide I.
- **Secondary Ion Mass Spectrometry (SIMS)** - IONTOF ToF SIMS 5 in negative polarity mode.
- CP signals recorded at 118.04 m/z (distinct from any tissue related molecule/fragments).

RESULTS

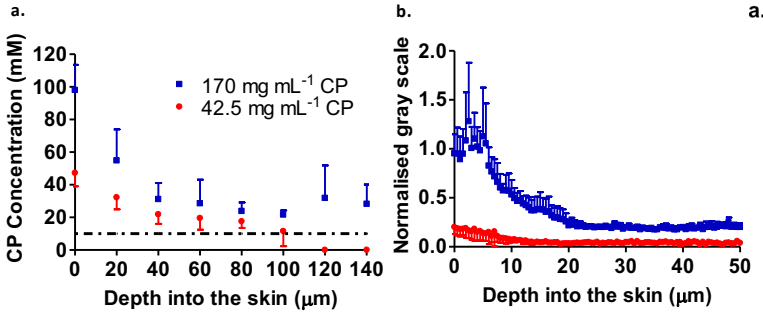
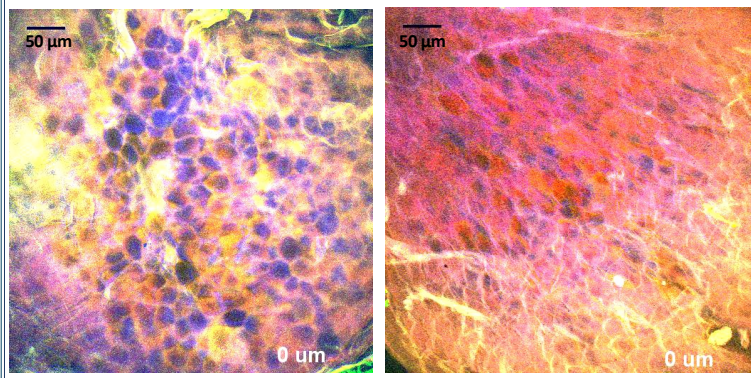


Figure 1: a. Confocal Raman spectroscopic quantification of CP uptake into skin as a function of depth from two formulations (mean + SD; n = 6 replicates of skin samples from 1 pig).

Dashed line = CP LOQ based on the Raman signal to noise ratio.

Figure 1: b. 'Top down' SRS: CP uptake into skin as a function of depth.



Animation 1: 'Top down' SRS imaging (CP = yellow, amide I = blue, CH_2 = red, SHG = green) of skin post application of the fully (left) and 25% (right) saturated CP formulations.

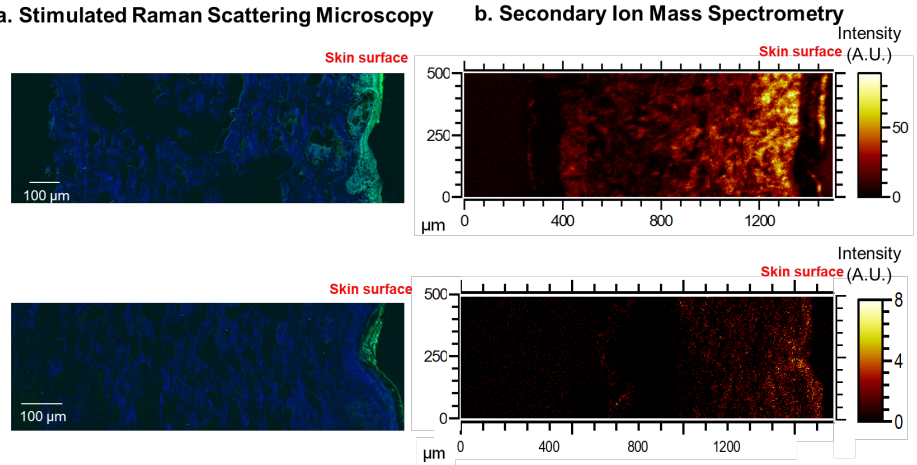


Figure 2: a. SRS (CP = green, amide I = blue) images of skin cross-sections post-application of fully (top) and 25% (bottom) saturated CP formulations.

Figure 2: b. SIMS images of the same skin cross-sections.

'Top-down' approach

- Confocal Raman and SRS imaging can detect CP to skin depths encompassing at least the epidermis (**Figure 1, Animation 1**).
- Depth profiles are consistent with and distinguish between the BA of CP from two different formulations.
- Additional work is needed to better understand and control sources of variability, and to minimise effect of background interference on Raman measurements.

Cross-section ('side-view') approach

- Cross-section imaging (SRS and SIMS) avoided sensitivity loss due to signal attenuation as a function of depth.
- Relatively high levels of CP in SC confirmed as well as greater uptake of chemical into epidermis from fully saturated solution (**Figure 2**).

CONCLUSIONS

- Raman spectroscopy is shown to be a potentially useful method to assess drug penetration into skin (and beyond the SC) and to compare performance of different formulations.
- Correlative imaging can provide evidence to support future application of Raman spectroscopy for the assessment of topical BA (and bioequivalence) *in vivo* .

ACKNOWLEDGMENT

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