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Monitoring of transdermal drug delivery in skin using the Renishaw Biological Analyser

Detecting the presence and depth of penetration of drug compounds in skin can prove challenging, current technologies fail to achieve adequate penetration through layers of dermal tissue. In this study, Raman spectroscopy using the Renishaw Biological Analyser - RA816 confirmed the presence of a topical compound in the epidermis and reticular dermis with high specificity and sensitivity.

Raman spectroscopy was also able to elucidate the alterations in the secondary structure conformation of protein peaks (α-helix to β-sheet) due to biochemical changes taking place during tissue remodelling following treatment.

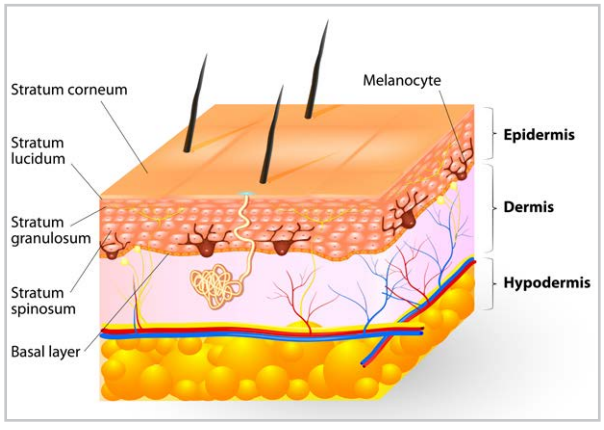
Case study

In a study, conducted with Miss Rubinder Basson from University of Manchester, Division of Musculoskeletal and Dermatological Sciences, the system demonstrated the presence and penetration depth of a compound in different skin tissue layers following application of a topical formulation.

This study consisted of a randomised blind clinical trial of 45 human subjects receiving an active topical (MEBO scar topical wound healing cream) against control with sequential punch biopsies. These were then evaluated using the Renishaw Biological Analyser as well as standard skin histology, qPCR gene expression, full-field laser perfusion imaging (elasticity and hydration), spectrophotometric intracutaneous analysis and optical coherence tomography.

The Renishaw Biological Analyser - RA816

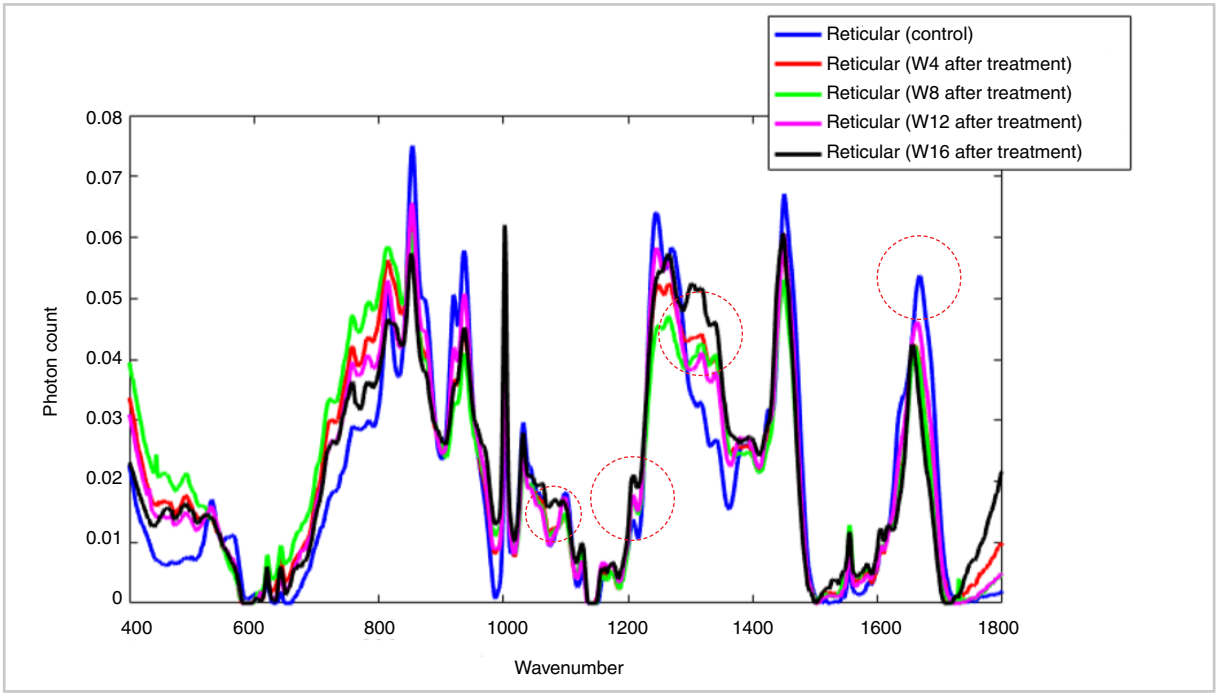
Patients cases were divided into four groups, each representing a time point. On 'Day 0', a 5 mm punch biopsy was taken under local anaesthetic from both upper inner arms to create a scar. At 'Day 14' the first biopsy measurements of the scar were taken (spectroscopic imaging, histology and qPCR) and subjects were given both active and control topicals. Subsequently, measurements on the four groups were taken at time points of 4, 8, 12 and 16 weeks respectively after daily use of the topical. Biopsies were either bisected and stored in formalin for later RNA analysis, or snap frozen by dry ice and stored at -80 °C so that they could be used for Raman spectroscopy. Snap frozen samples were cut in cross-sectional slices using a cryostat at 10 µm thickness and mounted on calcium fluoride slides for analysis using the Renishaw Biological Analyser.



Tissue layers of the human skin

Results: Transdermal delivery

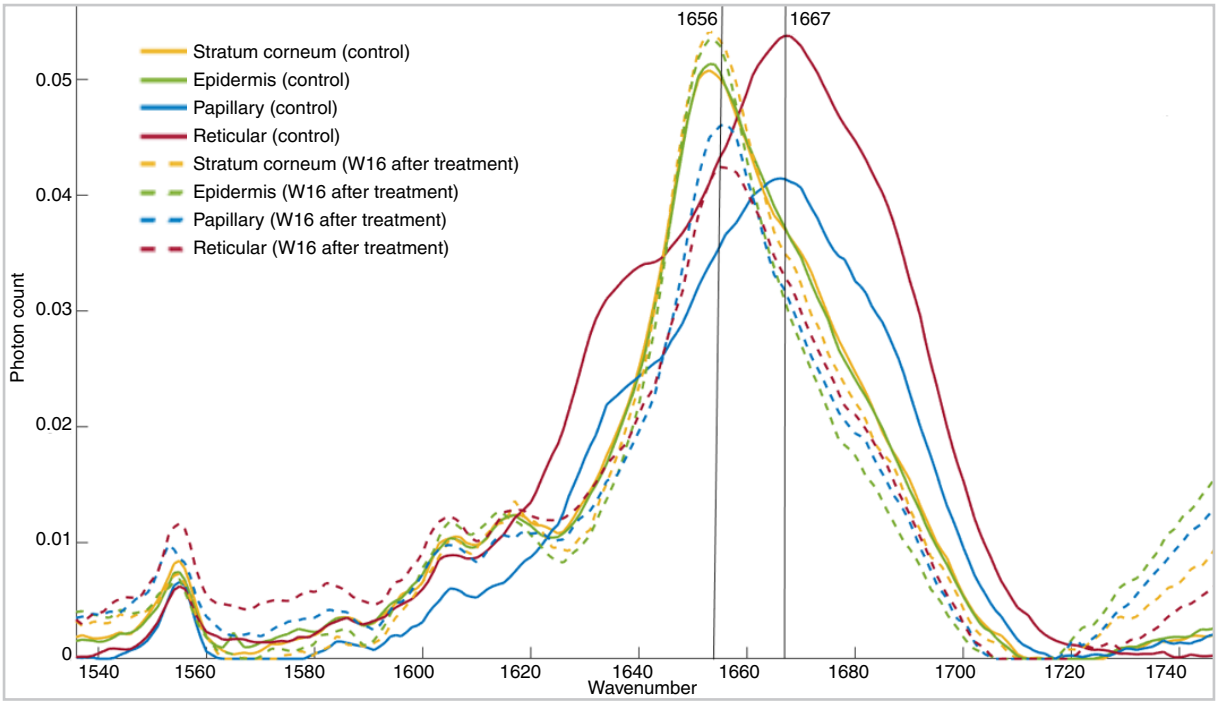
Raman spectroscopy confirmed the presence of the topical drug compound within 'normal skin' spectra. Within the scar biopsies, transdermal delivery of the active topical was evident in the epidermis and deep reticular dermis, and over sequential time points weeks 4-16, (98% and 99% specificity, 89% and 93% sensitivity, 96%, and 97% accuracy, respectively).



Meboscar™ compound was detected at higher level accumulation in the reticular dermis over sequential time points following treatment (day 0 to weeks 8-16) shown on the above plot at 1070 cm^{-1}, 1210 cm^{-1}, 1303 cm^{-1} and 1656 cm^{-1}

Results: Tissue remodelling

During the wound healing process, the levels of collagen I and III, fibronectin, and laminin all expectantly increase in raised dermal scar tissue. At week 16 in the active group, Raman spectroscopy showed a shift of the amide I peak from 1667 cm^{-1} (β-sheet contribution of elastin) to 1656 cm^{-1} (triple helix contribution of collagen types I and III) in the papillary and reticular layers of the dermis, highlighting the changes taking place during tissue remodelling following treatment. Non-invasive measurements of elasticity taken with a full-field laser perfusion imager, agreed with these Raman results, illustrating a reduction in elasticity measurements in week 16.

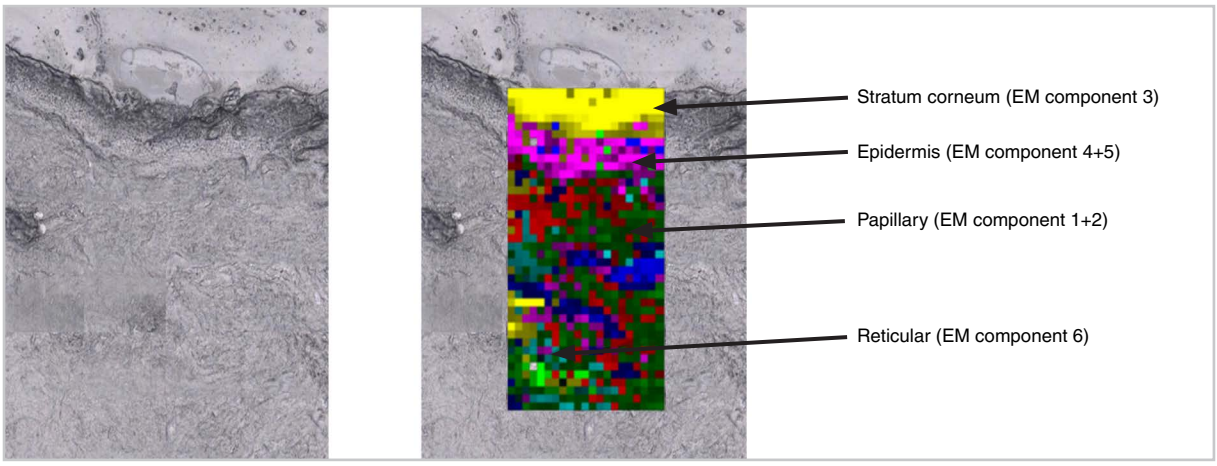


Amide I spectral region showing conformational change in protein structure from β-sheet contribution of elastin to the α-triple helix component of collagen. Observing effects of these compounds in wound-healing and tissue remodelling.

Results

The Renishaw Biological Analyser was able to:

- Confirm the diffusion of the topical active ingredient into different dermal layers
- Indicate a change in skin protein composition during the healing process
- Distinguish tissue layers by their overall chemical signatures – fluorescent or colorimetric labelling was not required
- Provide histological images without labelling



Tiled image of the unstained tissue section (left) and corresponding Empty modelling™ processed Raman image (right) overlaid on the unstained tissue section clearly showing the different tissue layers in the skin

The Renishaw Biological Analyser; the ideal Raman tissue imaging tool to meet the specific challenges of the clinical research environment

- Easy to use hardware operation and accessories targeted for bio-samples
- Optimal light microscopy performance for macro and high magnification
- Compact and transportable
- Optimised hardware for stable, repeatable, and high signal to noise spectral data acquisition
- Supports model and data transferability for disease and pathology classification on different systems
- Easy to use software that makes measurements accessible to all users without comprising performance
- StreamLine™ technology allows high speed mapping without causing sample damage

The Renishaw Biological Analyser is designed for Research Use Only (RUO) and not for use in diagnostic procedures.

Acknowledgements:
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References:
- Basson R, Isabelle M *et al. In vivo* functional testing of a topical using a three-tiered approach in human skin enables objective and quantitative evaluation of its role in skin scarring. Manuscript in preparation

A range of related Renishaw literature is available. Please ask your local Renishaw representative for more information.

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Renishaw manufactures a wide range of high performance optical spectroscopy products, including confocal Raman microscopes with high speed chemical imaging technology, dedicated Raman analysers, interfaces for scanning electron and atomic force microscopes, solid state lasers for spectroscopy and state-of-the-art cooled CCD detectors.

Offering the highest levels of performance, sensitivity and reliability across a diverse range of fields and applications, the instruments are designed to meet your needs, so you can tackle even the most challenging analytical problems with confidence.

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