In vivo and *ex vivo* analysis of skin structure: *a comparative study*

V. L. Newton[1,2], R.S. Bradley[3], P. Séroul[4], M. Cherel[4], C.E.M. Griffiths[1,2], A.V. Rawlings[5], R.Voegeli[6], R.E.B Watson[1,2] & M.J. Sherratt[7]

[1]Centre for Dermatology Research, Institute of Inflammation & Repair , Manchester Academic Health Science Centre, University of Manchester, UK; [2]The Dermatology Centre, Salford Royal NHS Foundation Trust, Salford, UK; [3]School of Materials, The University of Manchester, UK; [4] Newtone Technologies, Lyon, France; [5] AVR Consulting Ltd, Northwich, UK; [6]DSM Nutritional Products Ltd, Kaiseraugst, Switzerland; [7]Centre for Tissue Injury and Repair, Institute of Inflammation & Repair, Manchester Academic Health Science Centre, The University of Manchester, UK.

Introduction:

- Although histological techniques have historically been employed to characterise skin structure, these approaches commonly: i) require the collection of invasive biopsies, ii) induce mechanical damage (as a consequence of sectioning) and iii) are limited to 2D visualisation of inherently 3D structures.
- Micro-computed X-ray tomography (**microCT**) is a 3D imaging technique which is commonly applied to intact calcified tissues, but rarely used to visualise soft tissues.
- In contrast to these biopsy-based approaches, non-invasive *in vivo* imaging techniques not only avoid the discomfort and ethical implications of biopsy collection (thereby aiding recruitment), but also facilitate longitudinal studies. Currently however, their ability to image 3D volumes remains underdeveloped.

Aims:

- To compare the ability of *in vivo* (optical coherence tomography & confocal microscopy) and *ex vivo* (microCT & histology) to visualise and quantify key aspects of skin epidermal morphology.
- To determine if confocal microscopy and microCT can visualise skin structures, but primarily the epidermis, in 3D.

Methods:

- Male and female Caucasian volunteers were recruited into the study (*n*=10; 18-30 yrs old; 6 female, 4 male; University of Manchester Research Ethics No. 13268).
- An area of skin on the mid dorsal forearm (sun exposed) was imaged using:

i. A confocal laser microscope (**CM**; VivaScope® 1500; MAVIG GmbH, Germany; 5 stacks of sixty five 500 µm x 500 µm images taken at 3.05 µm intervals) *and*

ii. Optical Coherence Tomography (**OCT**; Swept Source OCT System, Thorlabs LTD, UK; 40 dB, 10 fields of view taken 0.2 mm apart, 5.9 µm/pixel).

- Biopsies were then taken of the imaged areas, fixed and either:

i. Wax embedded, sectioned and haematoxylin & eosin (H&E) stained for **histology** *or*

ii. Stained with Lugol's solution (I_2KI) for 18 hours before wax embedding & **microCT** imaging (Zeiss Versa XRM-510 system).

Result a): OCT and histology allow visualisation of the skin in cross section and direct comparison of epidermal thickness. Limitations in the resolution of OCT prevents the measurement of dermal papilla/rete ridge height. Epidermal thickness and dermal papilla/rete ridge heights measured using OCT & CM were greater than when measurements were taken from histological sections

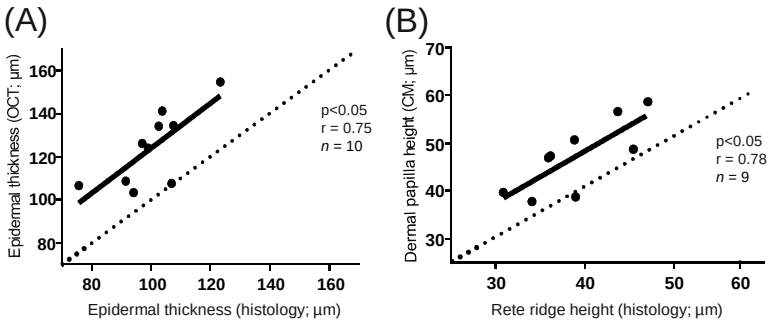

Fig 1: (A) Epidermal thickness measured using OCT gives larger values than when measured using histological sections stained with H&E, though values are well correlated. **(B)** Rete ridge/dermal papilla height measured using CM images gives higher values than when measured from histological sections. Pearson's r correlation; the solid line represents the line of regression; the dotted line marks thicknesses/heights of equal value.

Discussion:

Technique	Biopsy & fixation?	Sectioning required?	Epidermal resolution	Speed of initial image acquisition	3D?	Speed of 3D image creation and manipulation
Histology	Yes	Yes	High	Low	No	-
OCT	No	-	Med/Low	High	No~	-
CM	No	-	High (x & y), Med (z)	High	Yes	High*
MicroCT	Yes	No	High	Low	Yes	Low*

~ In this study, but is possible using some systems; * Where contrast is sufficiently high

- Measurement of epidermal skin structures using *in vivo* techniques compared well with those obtained using histology.
- Although histology is still the most robust method of skin visualisation, especially at high resolution, visualisation and analysis of the skin in 3D gives us new insight into the complexity of the skin.
- 3D reconstructions of CM and microCT images will allow studies to analyse the epidermis in terms of volume and 3D connectivity.
- Use of 3D imaging is likely to become more commonplace in the future as we further address the technical difficulties associated with these techniques in order to give us a more realistic window through which to view the skin.

Result b): CM images can be used to visualise dermal papilla in 3D without taking a biopsy, but segmentation of dermal papilla from CM images to allow this 3D visualisation was only possible where papilla were sufficiently distinct

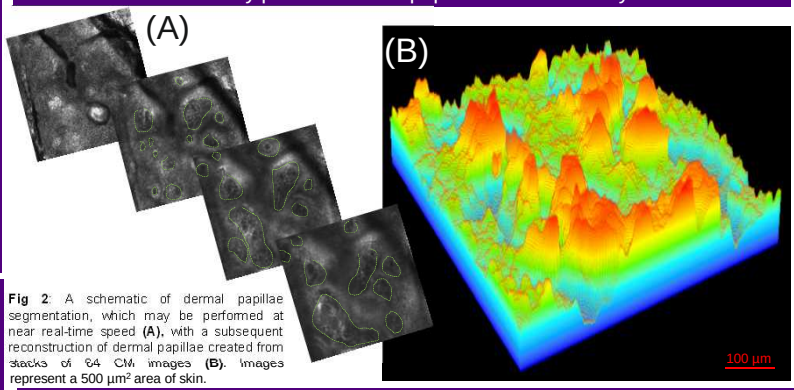

Fig 2: A schematic of dermal papillae segmentation, which may be performed at near real-time speed **(A)**, with a subsequent reconstruction of dermal papillae created from stacks of 64 CM images **(B)**. Images represent a 500 µm² area of skin.

Result c): Although requiring a biopsy, reconstructed microCT images allow the visualisation of many skin structures at high resolution, including hairs and their follicles, epidermal keratinocytes, and skin furrows. As whole biopsies can be imaged, tissue tearing and damage associated with sectioning is avoided

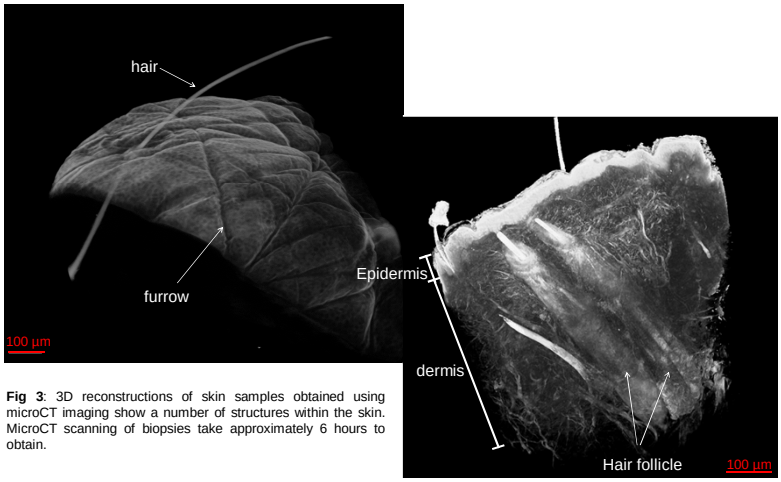

Fig 3: 3D reconstructions of skin samples obtained using microCT imaging show a number of structures within the skin. MicroCT scanning of biopsies take approximately 6 hours to obtain.

Result d): Differences in X-ray absorption allow the segmentation of the stratum corneum and epidermis from microCT scan images which can then be used in the quantification of a 3D section of these structures, for example, rete ridge height

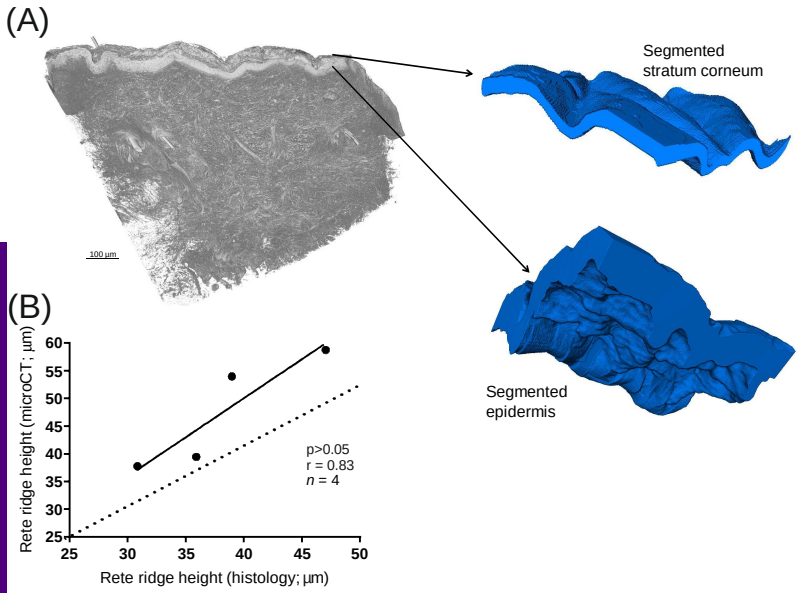

Fig 3: Volumes of the stratum corneum and epidermis can be segmented from 3D whole skin biopsy reconstructions (A). These may be used to extract structural information such as rete ridge height. Rete ridge heights obtained using microCT images are similar to those obtained using histology. Pearson's r correlation; the solid line represents the line of regression; the dotted line marks thicknesses of equal value; Stratum corneum represents a volume 250 µm³, the segmented epidermis a volume of 450 µm³.

Acknowledgements:

- MicroCT imaging was conducted at the Manchester X-ray Imaging Facility, which was funded in part by the EPSRC (grants EP/F007906/1, EP/F001452/1 & EP/I02249X/1).
- Thank you to DSM Nutritional Products for the loan of their VivaScope® CM microscope.
- Thank you to Graham Dinsdale and Andrea Murray for their help with and the use of their OCT equipment.
- This study was financially supported by DSM Nutritional Products Ltd.