

Non-invasive evaluation of the skin by reflectance confocal microscopy in experimental models of contact dermatitis

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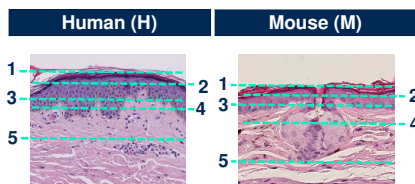
Introduction & Objective

- Reflectance confocal microscopy (RCM) is a technique that allows real-time examination of the skin at nearly histologic resolution
- It provides greyscale horizontal images based on the refractive index of cells and structures, being high in the case of keratin and melanin
- RCM has been proved valuable in the diagnosis of human dermatology diseases and research, however, studies in preclinical models are scarce
- Assessment of cutaneous tolerance or sensitization of compounds intended for the topical route is still performed in animals despite efforts to replace them by in-vitro methods
- Non-invasive techniques such as RCM could aid in reducing and refining the number of animals used in this type of studies

The objective is to investigate the ability for detecting allergic and irritant contact dermatitis (CD) by RCM in two murine models, after identifying characteristic features in healthy skin

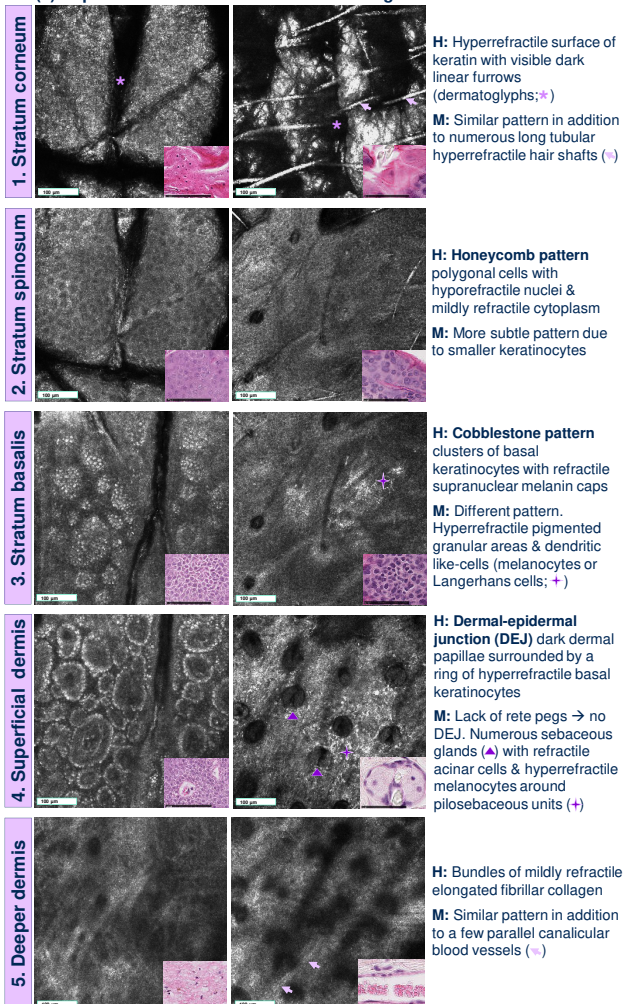
Confocal features in healthy skin

- Interpretation of the images in mice was learned by comparing with well characterized features described in human skin, as well as by correlating with horizontal H&E sections



Note the thin epidermis and lack of rete pegs in murine auricular skin compared to human skin

(#) Representative location of confocal images

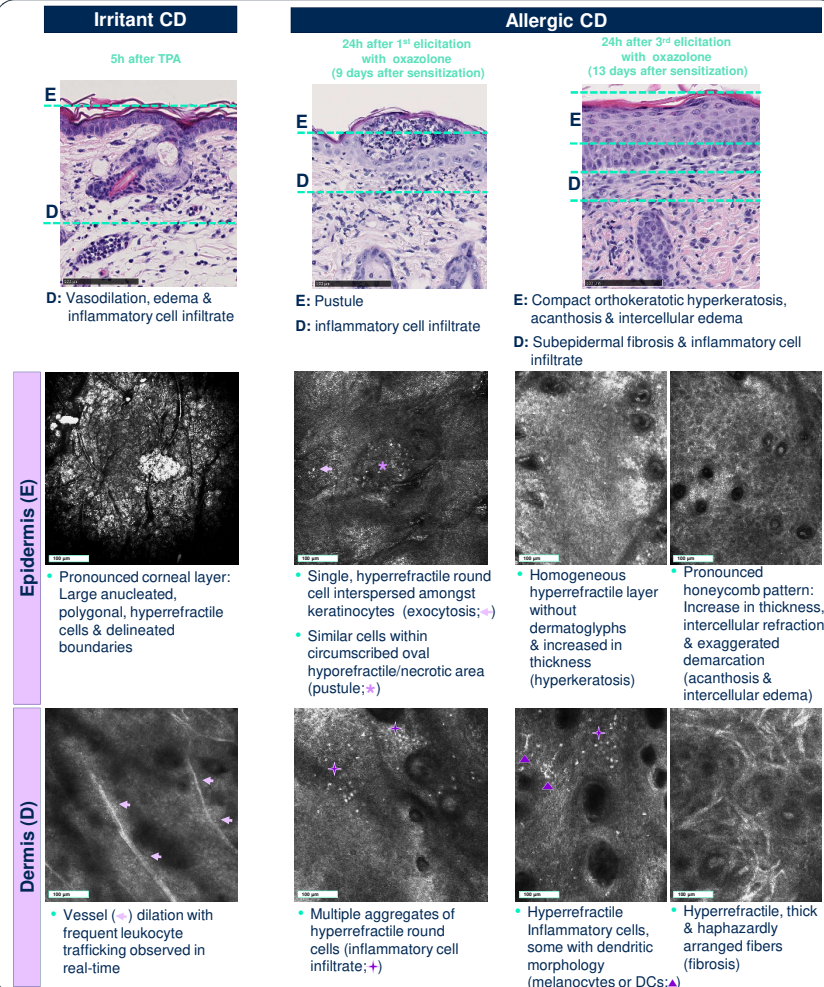


Methods

- Allergic CD was elicited in the depilated dorsal skin of mice 9-13 days after sensitization by repeated treatment with 0.5% oxazolone, whilst a single topical application of 0.01% TPA induced irritant CD in the auricle
- VivaScope® 3000 or handheld VivaScope® 1500 (MAVIG, Germany) were used to take horizontal optical sections from relevant skin layers up to the maximum imaging depth of 250 µm
- Imaging was performed on healthy or lesional skin of 6-8 weeks-old, male C57BL/6JRj mice and the forearm of a healthy human volunteer at Almirall
- Mice were anesthetized prior and during the imaging, and were subsequently euthanized. A biopsy was collected and processed into horizontal or vertical H&E sections.
- Procedures were approved by the Animal Experimentation Ethics Committee of Almirall & the competent authority body in accordance with EU Directive 2010/63/EU and following ARRIVE guidelines



Major cutaneous findings in murine models of contact dermatitis



Conclusion

- ❖ This is the first comprehensive description of the major architectural and cellular confocal features of healthy and lesional murine skin, to our knowledge
- ❖ RCM allowed the *in-vivo* evaluation of acute to chronic-active cutaneous changes characteristic of contact dermatitis, even at subclinical levels
- ❖ Its non-invasive nature will be useful to assess the progression and reversibility of findings in the same treatment area and animal, in future sensitization and cutaneous tolerance studies

The application of RCM represents a novel approach for the *in-vivo* screening for irritation or sensitization of dermal drugs, while complying with the "3Rs"

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References

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