

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 realtime 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

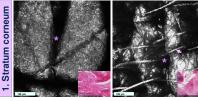
The objective is to investigate the ability for detecting allergic and irritant contact dermatitis (CD) by RCM in two murine models, after identifying characteristic features in healthy skin

Confocal features in healthy skin

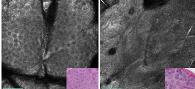
Interpretation of the images in mice was learned by comparing with well characterized features described in human skin, as well as by correlating with horizontal H&E sections

epidermis and lack of rete pegs in murine

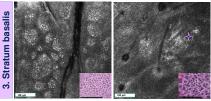
(#) Representative location of confocal images



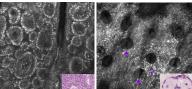
- H: Hyperrefractile surface of keratin with visible dark (dermatoglyphs;*)
- M: Similar pattern in addition to numerous long tubular hyperrefractile hair shafts (*)



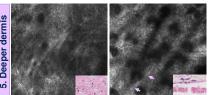
- H: Honeycomb pattern polygonal cells with hyporefractile nuclei & mildly refractile cytoplasm
- M: More subtle pattern due to smaller keratinocytes



- H: Cobblestone pattern clusters of basa keratinocytes with refractile supranuclear melanin caps
- M: Different nattern Hyperrefractile pigmented granular areas & dendritic like-cells (melanocytes or Langerhans cells: +)



- H: Dermal-epidermal junction (DEJ) dark dermal papillae surrounded by a ring of hyperrefractile basal keratinocytes
- M: Lack of rete pegs → no DEJ. Numerous sebaceous glands (A) with refractile acinar cells & hyperrefractile melanocytes around pilosebaceous units (+)



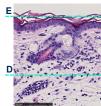
- H: Bundles of mildly refractile elongated fibrillar collagen
- M: Similar pattern in addition to a few parallel canalicular blood vessels (**)

- · 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 anesthesized 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

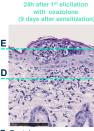


Major cutaneous findings in murine models of contact dermatitis

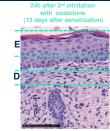
Irritant CD



D: Vasodilation, edema & inflammatory cell infiltrate



D: inflammatory cell infiltrate



Allergic CD

E: Compact orthokeratotic hyperkeratosis. acanthosis & intercellular edema

D: Subepidermal fibrosis & inflammatory cell



Pronounced corneal layer Large anucleated polygonal, hyperrefractile cells & delineated

Vessel (-) dilation with

trafficking observed in

frequent leukocyte

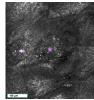
real-time

E

Epidermis

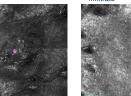
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Dermis (

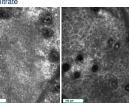


Single, hyperrefractile round cell interspersed amongst keratinocytes (exocytosis;-)

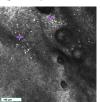
Similar cells within circumscribed oval hyporefractile/necrotic area



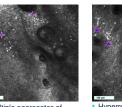
without dermatoglyphs & increased in (hyperkeratosis)



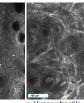
Homogeneous hyperrefractile layer honeycomb pattern: intercellular refraction & exaggerated demarcation



Multiple aggregates of hyperrefractile round cells (inflammatory cell



Hyperrefractile Inflammatory cells some with dendritic morphology (melanocytes or DCs; A)



Hyperrefractile, thick & haphazardly arranged fibers

intercellular edema)

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'

Authors thank Joan Mañé, Gloria Aniorte, Lluís Boix and Jose L.

- González S. et al. 2008. Informa Healthcare
- Swindells et al. J Am Acad Dermatol, 2004; 50:2, 220-228

Stratum

Superficial