

## Hydration (Humidity?)-dependent *ex vivo* corneocyte maturation and the importance of transglutaminase and 12R-lipoxygenase activities.

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Corneocyte protein envelopes (CPE) are assembled in the late stage of keratinocyte differentiation and coated covalently with lipids to form the corneocyte lipid envelope (CLE). In this process 12R-lipoxygenase (12R-LOX) and other enzymes process linoleoyl acylceramides before transglutaminase 1 (TG) attaches the omega-hydroxy fatty acid-containing ceramides covalently to the CPE while also forming isodipeptide bonds. This work demonstrated a) the effect of relative humidity on *ex vivo* cornified envelope (CE) maturation, b) the effect of a mixture of broad spectrum protease inhibitors (PI) on TG activity and c) the impact of 12R-LOX activity in optimal *ex vivo* maturation conditions in the first and ninth tape strippings of the photo-exposed (PE) cheek and photo-protected (PP) post auricular sites from healthy Chinese volunteers (n=12, age 25 ± 3 years). CE maturity was assessed by CE rigidity and CE hydrophobicity per unit of CE surface area. Irrespective of tape stripping depth, PE samples had an increase in CE rigidity to the same extent as more mature PP samples, but such improvement was lacking for CE hydrophobicity. *Ex vivo* CE maturation was optimal at 70% RH. CE in the presence of a 12R-LOX antibody, to block its activity, showed enhanced CE rigidity but was reduced by the TG inhibitor LDN-27219. CE hydrophobicity remained unchanged irrespective of *ex vivo* conditions. The second and eighth tape strippings were examined in order to understand the effect of RH on the activity of TG. High hydration diminished TG activity more than the commercially available inhibitor LDN-27219. Furthermore, a protease inhibitor mix was able to overcome the negative effect of over-hydration. Both enzymes showed a similar pattern of activity in both anatomical sites where PP samples were enzymatically more active especially in the deeper SC layers. This study highlights the roles of 12R-LOX and TG activities in CE maturation.