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Development and validation of tissue-engineered human skin equivalents to detect stinging potential

Introduction: There are no physical manifestations that define skin sensitivity and a subjective diagnosis is made based on sensory indicators including burning, prickling and itching. Adverse skin sensation in response to topical products is a common reason for poor treatment compliance, limiting the use of dermatological products. The stinging test is an *in-vivo* procedure that is commonly used to determine the tolerability of a given chemical on sensitive skin and is regularly used by industry to test compounds that exhibit soothing properties or inhibit stinging. However, no measurable outcomes are recorded and the cellular and molecular mechanisms driving skin sensitivity are unknown.

Aim: To develop a novel tissue-engineered skin equivalent that is able to predict stinging potential *in-vitro*.

Methods and results: Human skin equivalents (HSE) were generated by embedding primary or immortalised dermal fibroblasts in a collagen hydrogel and culturing hTERT-immortalised skin keratinocytes on top at an air-to-liquid interface for 14d. Stinging reagent lactic acid (LA; 5%), or cosmetic chemicals methyl-paraben (methyl-P, 0.2%) cocamide diethanolamine/monoethanolamine (Co-DEA/MEA; 2%) were added topically to HSE or *ex-vivo* skin explants for 24h. Lactate dehydrogenase release and histological analysis revealed a significant increase in HSE cytotoxicity after incubation with vehicle control (dH₂O) and LA compared to untreated controls but no effect was observed in *ex-vivo* skin or for the other compounds tested. Permeability barrier function measured using transepithelial electrical resistance (TEER) found no significant differences compared to untreated controls in HSE, whereas LA treatment significantly reduced TEER in *ex-vivo* skin. Identification of a 'stinging' molecular signature was assessed using a 22 gene-panel by qPCR. In *ex-vivo* skin, LA treatment resulted in a significant fold increase in AFT3, F2RL2, FN-1, STAT1, HMGB2, IL1 β , MAP3K8 and TAC-1 and a fold decrease in MMP-3, whilst methyl-P only caused a significant fold decrease in MAP3K8. Co-DEA induced fold increases in F2RL2 and HMGB2 and a fold decrease in MMP-3 whilst treatment with Co-MEA significantly increased expression of IL-1 α and again a fold decrease in MMP-3. In HSE, LA treatment also resulted in a significant fold increase in STAT1, MAP3K8, TAC-1 expression, as well as HSP1A and MMP-3. No significant observations were made with methyl-P or Co-MEA treatment, however, HSP1A expression significantly increased with Co-DEA treatment. A fold decrease in CALCA expression was observed for all chemical compounds tested. HMGB2, TAC-1, IL-1 β and IL-6 levels in conditioned medium from HSE and *ex-vivo* skin were determined by ELISA. LA treatment of HSE significantly increased TAC-1 release compared to dH₂O controls, reflecting what was observed at the gene level. A significant increase in IL-6 was observed for both Co-DEA and Co-MEA.

Summary: This study developed *in vitro* and validated in *ex-vivo* skin evidences a 'stinging' gene signature, with translation for use in future chemical testing of skin.