VitroScreen
excellence in *in vitro* science

REPEATED EXPOSURES TO HARD WATER AND SURFACTANT IMPAIRS THE EPIDERMAL BARRIER DEVELOPMENT IN VITRO

Jennifer BOURLAND[1], Francesco RANZINI[1], Alessandra SEMENZATO[2] Gianni BARATTO[2] and Marisa MELONI[1]
[1] VitroScreen Srl, Milan, Italy; [2] UNIR&D Srl, Padua, Italy
e-mail: jennifer.bourland@vitroscreen.com

INTRODUCTION & AIM

The development and organization of human skin barrier function after birth is a delicate adaptive process leading to a mature *stratum corneum* (SC) at the end of infancy. Before SC complete formation, skin is considered as immature and clinical data have demonstrated its fragility and sensitivity towards the domestic water hardness. In combination with surfactant such as sodium lauryl sulfate or sodium dodecyl sulfate (SDS), water hardness can alter barrier function in infants, leading to an increased risk of atopic dermatitis. To gain a better understanding of the effect of hard water and surfactant on skin barrier development as it occurs during infancy, we developed an *in vitro* model allowing to quantify the deleterious effects of exposure to hard water (HW) alone or in presence of surfactants on barrier function structural and functional parameters.

HYPOTHESIS

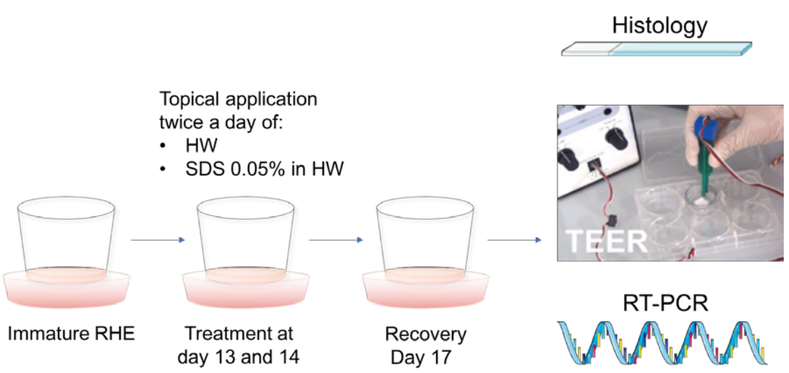
Repeated exposure to a combination of hard water and SDS induces an impairment of the barrier functionality and structure of an *in vitro* human immature reconstructed epidermis mimicking the effect of surfactant exposure during infancy.

MATERIAL & METHOD

Rationale of surfactant exposure:

$$Concentration = \frac{Daily\ product\ application}{Total\ skin\ surface\ area} = 0.001\ g/cm^2$$

As only a small proportion of the daily hygiene products remains on the skin, 1% of the daily product application dose was applied two times a day (equivalent to SDS 0.05 %)



Multiple Endpoint Approach

Figure 1: Evaluation of hard water and surfactant effect on RHE. To evaluate the effect of HW and surfactant on young skin, RHE (Episkin, France) were received at day 12 when the epidermis is still considered immature and in differentiation. After an overnight recovery, tissues were exposed twice a day to Hard water **HW** (ultrapure water with 400 mg/L (40 °F) calcium carbonate) or to **0.05% SDS** in hard water (**SDS + HW**) to mimic daily exposure to surfactants from day 13 to day 14 and then after 2 days recovery period, at day 17. Trans-epithelial electrical resistance was measured to quantify barrier permeability and properties. RHEs were then collected for histo-morphology, IF and qRT-PCR analysis.

➢ **Immature RHE (from Day 12) were used to mimic new-born or young epidermis.**

RESULTS

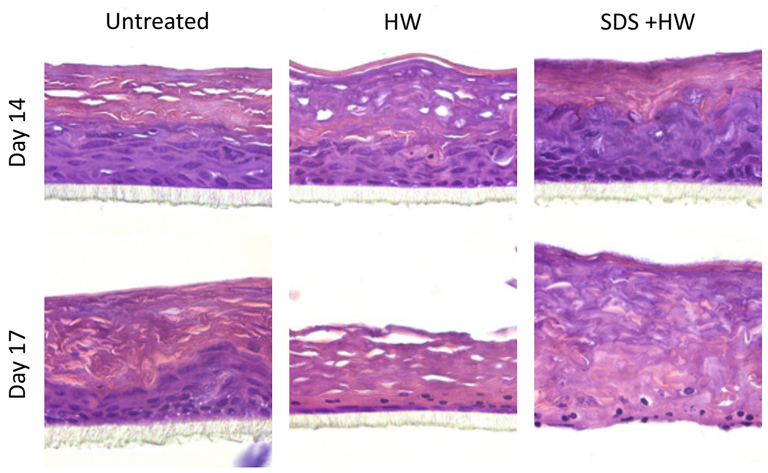


Figure 2: Modification of epidermal morphology and viability after HW and surfactant exposure. Hematoxylin and eosin staining of untreated RHE, hard water exposed RHE (HW) and RHE exposed to SDS combined with hard water (SDS +HW). Tissues were collected at day 14 (immediately after the 4th exposure) and at day 17 (after 2 days recovery).

➢ **The combination of SDS and HW alters epidermal morphology and differentiation.**

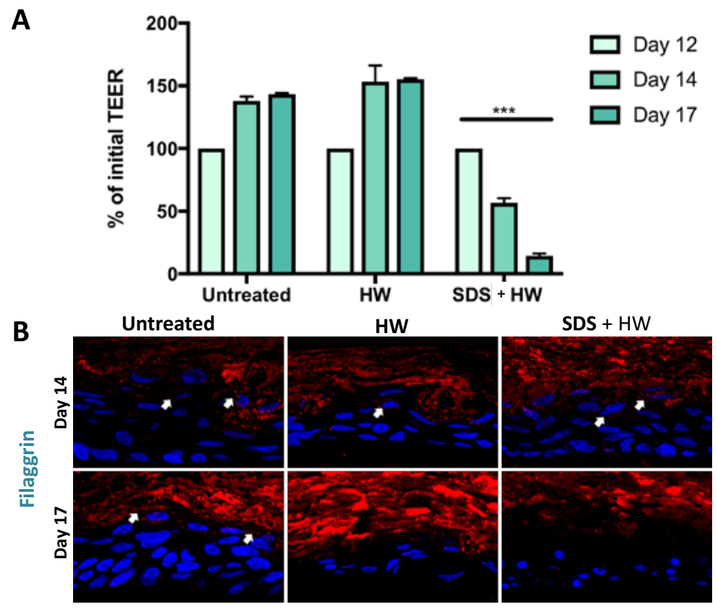


Figure 3: Modification of the barrier function.
(A) Measurement of trans-epithelial electrical resistance (**TEER**) of the RHE before (day 12) and after treatment (day 14) as well as after recovery (day 17). TEER values were significantly decreased in presence of SDS+HW, (2way ANOVA with multiple comparison, $p \leq 0.0001$).
(B) **Filaggrin** immunostaining (red signal) shows a decrease of filaggrin in the granular layer after HW and SDS+HW exposure (granules indicated by white arrows).

➢ **Barrier function is not severely modified by HW alone but rather by a combination of HW and surfactant.**

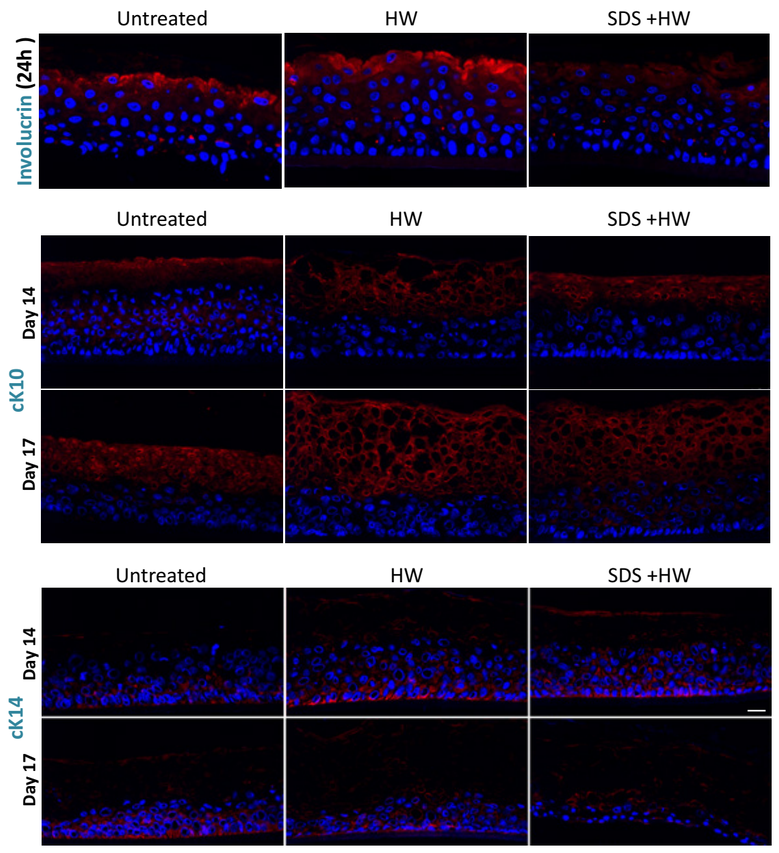


Figure 4: Epidermal differentiation biomarkers. Nuclei were counterstained with DAPI (blue signal) while involucrin, cK10 and cK14 are indicated by a red signal. Scale bar: 25 µm.
Involucrin immunostaining after 24h exposure to HW or SDS +HW and Keratin 10 (cK10) and Keratin 14 (cK14) immunostaining at day 14 and day 17.
Involucrin had completely disappeared after 24h in presence of SDS + HW.
cK10 and cK14 localization were not modified by HW/HW+SDS after 2 days exposures but on the contrary a reduced expression of both biomarkers were observed at day 17, indicating a trouble in the differentiation process and in the final barrier development.

➢ **The combination of SDS + HW modifies epidermal differentiation and barrier development.**

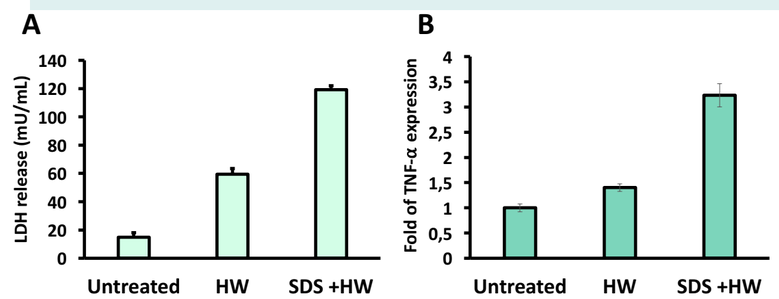


Figure 5: Keratinocyte inflammatory response to exposure to hard water and surfactant. (A) Quantification of LDH release (Cytotoxicity Detection Kit-LDH, Roche) in conditioned medium of RHE exposed to HW or to SDS+HW after 24h and two topical applications. (B) TNF-α expression at day 14 indicates a strong pro-inflammatory effect of SDS+HW.

➢ **SDS in hard water induces cellular toxicity as well as an inflammatory response.**

CONCLUSION

➢ **Immature RHE (day 12-14 of in vitro differentiation) is a good model to mimic the barrier fragility during infancy.**

➢ **HW (400 mg/L calcium carbonate - 40 °F) itself has demonstrated a moderate inflammatory effect on keratinocytes and the main deleterious effects were observed after repeated exposure to 0.05% SDS+HW : increased cytotoxicity, reduction of barrier function as observed by TEER value decrease, reduction of filaggrin and involucrin expression and alteration of epidermal differentiation (reduced expression of cK10 and cK 14 compared to untreated RHE).**