Skin capacitance imaging and corneosurfametry. A comparative assessment of the impact of surfactants on stratum corneum

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Silicon image sensor (SIS) technology was recently introduced as an innovative tool (SkinChip®, L’Oréal) providing sensitive imaging of the skin capacitance. This method can detect discrete focal variations in skin surface hydration, and thus early discrete manifestations of skin irritation induced by surfactants. In the present in vivo study, 2 neat and diluted shampoos, and 5% and 10% sodium laurylsulfate solutions were tested on human skin. Each surfactant solution was gently rubbed on the skin using wet hair wicks mimicking the casual use of a shampoo on the scalp. Clinical and SIS evaluations were carried out. In addition, the same products were tested using the ex vivo corneosurfametry bioassay performed on human stratum corneum (SC) harvested by cyanoacrylate skin surface stripplings. The colourimetric index of mildness (CIM) was measured on these samples. The product reactivity with the SC was recognized by darker skin capacitance images, and by both lowered SkinChip®-generated values and lowered CIM values. The extent in changes varied according to the nature of the test products and their concentrations. The SkinChip® image changes likely corresponded to the acute surfactant-induced water swelling of the corneocytes. Skin capacitance imaging and corneosurfametry allow to disclose discrete surfactant-induced alterations of corneocytes.

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Surfactants present in hygiene and skin care products are in part adsorbed at the skin surface (1), and they can also permeate the stratum corneum (SC) where they interact with proteins and lipids (2). In vitro studies have revealed a number of physico-chemical interactions between corneocytes and surfactants (2–4). One of the earliest events following surfactant-induced protein denaturation is perceived as corneocyte swelling (5–8). This condition leads to a paradoxical and transient SC hydration following surfactant challenge in vivo (8–10). The structure and physical properties of the SC can be altered profoundly by environmental factors (11, 12), particularly following prolonged contact with anionic surfactants (4–10). As a consequence, minimal to severe irritation may develop with variable severity (10, 13). Full-blown lesions show inflammatory erythema, increased transepidermal water loss, altered cutaneous microrelief, increased SC roughness and erratic desquamation (13–15). Several instrumental methods can indirectly assess some specific aspects of cutaneous irritation on human SC (4, 9, 10, 16–19). In particular, the SC water content can be assessed in vivo using devices measuring changes in electrical properties of skin at different frequencies and at different depths inside the SC (10, 16–19).

This study was conducted in vivo in order to assess early and subclinical corneocyte swelling following contact of surfactant solutions with human SC. The new silicon image sensor (SIS) technology (19–22) was used on human skin. This method provides information about SC surface topography, SC collection of transepidermal exudate and SC hydration. The skin capacitance imaging method has already been compared to the casual capacitance method using the Corneometer® device (C + K electronic, Cologne, Germany). A statistically linear correlation was found between the mean SkinChip® values and the Corneometer® values (20). Skin capacitance imaging has already been used to explore various physiopathological conditions (8, 23–29). The ex vivo corneosurfametry bioassay (30–35) was also performed. It represents a predictive means for testing the potential severity of surfactant interaction with human SC.
The aim of this study was to compare corneosurfametry data with skin capacitance imaging for assessing the proclivity to develop mild surfactant-induced SC alterations. Neat and diluted shampoos, and sodium laurylsulfate (SLS) solutions were used as test products.

Materials and Methods
This double-blind study was performed in accordance with the Declaration of Helsinki and its subsequent amendments. It was approved by the local Ethic Committee. Informed consent was obtained after the nature of the procedures had been fully explained.

Products
Two shampoo formulations were tested. Formulation A was a shampoo containing ammonium laureth sulfate and ammonium lauryl sulfate, it was tested \textit{in vivo} as a neat product and at a 50\% v/v dilution. Formulation B was a mild shampoo especially designed for children. It contained sodium laureth sulfate and cocoamido propyl betaine. Due to its mildness, it was only tested \textit{in vivo} as a neat product. Water solutions of 5\% and 10\% SLS were also tested.

Experimental design
The 3 shampoo-based formulations (neat shampoo A, 50\% shampoo A, neat shampoo B) were randomly applied to the volar aspect of the forearms of 10 healthy volunteers. Two 6 cm$^2$ areas were delimited on each forearm. Products were applied for 1 min while massaging with a wet hair wick. Products with their respective hair wicks were left on the test sites for 5 min. One control site was secured with only a water soaked hair wick deposited on the skin site. All sites were then rinsed with tap water and gently patted dry. The 5\% and 10\% SLS solutions were tested in a second series of 10 healthy volunteers. The \textit{in vivo} procedure was identical to that used for the shampoos.

Assessments
Clinical assessments of erythema were performed by the investigators on a 10-level grading scale. Sensory self-assessments were also performed and rated on a 10-level grading scale. \textit{In vivo} instrumental assessments (SkinChip$^{\text{TM}}$ device, L’Oréal, Paris, France) aimed at providing quantitative skin capacitance imaging. The SkinChip$^{\text{TM}}$ probe was composed of an array of 360 \times 256 microsensors located on a 18 \times 12.8 mm surface (20). Each sensor-cell contained an active capacitive feedback circuit whose effective feedback capacitance was modulated by the close contact of the probe with the skin. The SkinChip$^{\text{TM}}$ sensor generated detailed 50 µm pixel images of the skin in less than one-tenth of second. The resulting capacitance map corresponded to the relative hydration of the tested surface. The SkinChip$^{\text{TM}}$ measurements were obtained after firmly applying the probe onto the test sites for 5 s. For testing the shampoo formulations, the observations were carried out 2, 7 and 12 min after wiping and patting dry the test sites. The more hydrated portions of the SC appeared as darker pixels corresponding to decreased SkinChip$^{\text{TM}}$-generated values. For the SLS solutions, the observations were made 2 min after wiping and patting dry the test sites. Computerized analytical assessment was performed in order to quantify the heterogeneity in the multiple capacitance-based measurements. The test products were also tested using the corneosurfametry bioassay. For that purpose, series of 6 cyanoacrylate skin surface strippings (CSSS) were harvested from the intact forearm skin in healthy volunteers. A 2-h exposure time was respected between the shampoo formulations and the CSSS. Due to the stronger SLS reactivity with the SC, the exposure time between the SLS solutions and the CSSS was limited to 6 min. Interactions between the SC and the surfactants were conveniently assessed by reflectance colourimetry (Chroma Meter CR200; Minolta, Osaka, Japan) after a 1-min controlled staining with a toluidine blue-basic fuschin dye solution. The $L^*$ and Chroma $C^*$ values were recorded to derive the colourimetric index of mildness (CIM) corresponding to the difference between the $L^*$ and Chroma $C^*$ values. The CIM median values were calculated for each test product at each dilution. It is acknowledged that the CIM value increases with the potential irritancy of a product. For each subject and each test site, the mean value was calculated for the multiple SkinChip$^{\text{TM}}$-generated capacitance-based values. Data gathered in each group of subjects were expressed as medians and ranges of the individual values. Between-product comparisons were made using the paired non-parametric Friedman test followed by the Dunn test. A $P$-value <0.05 was considered significant.

Results
Shampoo formulations
No significant difference was yielded in the sensory self-evaluations gathered during testing the
different shampoo formulations. A faint erythema developed in 8 of 10 panelists at the site tested with the undiluted shampoo A, while only 3 of 8 panelists reacted at the sites tested with the diluted shampoo A and the undiluted shampoo B. A significant difference \((P < 0.008)\) in erythema was yielded between the neat shampoo A (median: 0.95) and the water control (median: 0.00). No significant difference in erythema was found between the 2 skin sites corresponding to the diluted shampoo A (median: 0.00) and the next shampoo B (median: 0.00).

The SkinChip\textsuperscript{R} generated data about the shampoos are presented in Fig. 1. At the 2-min evaluation time, significant differences \((P < 0.05)\) were yielded between the values gained for the neat shampoo A and any of the other test shampoos. The hydration values obtained with the neat shampoo A corresponded to grey levels darker than for the other formulations. 12 min after the end of shampoo exposure no more significant difference persisted between the SkinChip\textsuperscript{R} values corresponding to the 3 formulations (Fig. 1).

A significant difference \((P < 0.001)\) was yielded between the corneosurfametry CIM values of the shampoo A (median: –16.3) and the shampoo B (median: 11.9).

**SLS formulations**

Using SLS solutions, the median SkinChip\textsuperscript{R}-derived values was lower for the 10% concentration (median: 179.5) than for the 5% solution (median: 193.5) which was itself lower than for the control water solution (median: 234.5). Significant differences were obtained between the 5% SLS and control \((P < 0.001)\), and between the 10% SLS and control \((P < 0.05)\). The corneocyte swelling appeared proportional to the SLS concentration.

The median corneosurfametry CIM values obtained for the 5% SLS solution (12.0) was significantly \((P < 0.001)\) higher than the median CIM of the 10% SLS solution (8.0) (Fig. 1).

**Discussion**

The daily use of surfactant-based products, even the milder ones, can induce cutaneous irritation (4, 14, 32, 36). Some individuals are more susceptible to develop such a reaction (10, 13). Many predictive in vitro and in vivo methods have been designed to assess the irritation potential of surfactants (4, 9, 10). The utility of SkinChip\textsuperscript{R} technology for differentiating shampoos exhibiting different irritation potential was previously highlighted while testing the products under occlusion patches (8). This corresponded to a more aggressive condition of use than in the current experiment. The present study was designed to rate the corneocyte swelling induced by a non-exaggerated use of the shampoos. The SkinChip\textsuperscript{R} grading value of shampoo-induced corneocyte damages was confirmed by the SLS experiment.

The non-discriminative felt perception between the tested shampoos was not a surprise as the products were applied under gentle conditions. By contrast, the erythema assessment clearly indicated that the neat shampoo A was more aggressive than the neat shampoo B. This difference was confirmed by the SkinChip\textsuperscript{R} method and the corneosurfametry bioassay.

The kinetics of SC changes following surfactant challenge is complex (37). An increase in SC hydration is present at the end of exposure to surfactants and usually exhibits a rapid reversibility (9). An increased skin conductance persisting 1 h after removing 1% SLS patch-tests left in place for 24 h has been reported (38). This is at variance with another study where electrometric values were not modified significantly when readings were made 1 h after removal of single or iterative 2-h SLS patch-tests (16). In this experiment, a later reading after 24 h showed a significant decrease in electrometric values. These findings were consistent with other studies indicating a decreased hydration state up to 3–6 days (39, 40).

In the present study, skin capacitance imaging showed the earliest SC changes at the test sites. The process was revealed by darker pixels corresponding to water-enriched corneocytes in contact with the probe. This aspect was probably related to the corneocyte swelling resulting from the transient intracellular accumulation of unbound water. Corneocyte swelling is indeed the net result of electrostatic repulsive forces. As the substrate expands and the tertiary structure is disrupted,
additional molecular sites become available for surfactant binding (5). Swelling is reversible by removal of the surfactant when the protein structure has not been permanently denatured. By a matter of fact, the darker aspect quickly disappeared under soft testing conditions. A 6-min exposure time was selected at the corneosurfametry bioassay to compare the 2 SLS concentrations. Indeed, SLS being aggressive for the SC, the regular 2 h-exposure time in the corneosurfametry bioassay would have been too long to discriminate the effects of the 5% and 10% SLS concentrations (31).

It is generally acknowledged that a clear, although not always stringent relationship exists between SC swelling and skin irritation (5). In this study, the corneosurfametry bioassay confirmed that the more irritant products induced a more intense corneocyte swelling. The results of both corneosurfametry and SIS technology were in line for evaluating the potential aggressiveness of the surfactant-based products, either shampoos or SLS solutions. As expected, SIS technology showed higher grey levels for the more concentrated SLS solution, which corresponds to a more intense swelling of the corneocytes.

In conclusion, skin capacitance imaging appears to be a reliable tool in the evaluation of the irritative potential of a surfactant solution in non-exaggerated conditions of use. This method can be added to the list of many in vitro and in vivo predictive methods presently available for rating the potential irritancy of surfactants (4, 9, 10, 16–18, 41, 42). Searching for more sensitive methods remains one of the goals in this field of research. However, no single test method correlates precisely with the variety of events that occur during the interaction between surfactants and the skin. Hence, a generally acknowledged method evaluating skin compatibility of surfactant does not exist so far. Nevertheless, skin capacitance imaging appears workable for testing surfactant-based products under open or occlusive conditions.

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References


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