

Anti-irritant and anti-inflammatory effects of glycerol and xylitol in sodium lauryl sulfate-induced acute irritation

Effects of polyols in irritant contact dermatitis

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Abstract

BACKGROUND: Glycerol is known to possess anti-irritant and hydrating properties and previous studies suggested that xylitol may also have similar effects.

OBJECTIVE: Our aim was to study whether different concentrations of these polyols restore skin barrier function and soothe inflammation in sodium lauryl sulfate (SLS)-induced acute irritation.

METHODS: The experiments were performed on male SKH-1 hairless mice. The skin of the dorsal region was exposed to SLS (5%) for 3h alone or together with 5% or 10% of glycerol, respectively. Further 2 groups received xylitol solutions (8.26% and 16.52%, respectively) using the same osmolarities, which were equivalent to those of the glycerol treatments. The control group was treated with purified water. Transepidermal water loss (TEWL) and skin hydration were determined. Microcirculatory parameters of inflammation were observed by means of intravital videomicroscopy (IVM). Furthermore, accumulation of neutrophil granulocytes and lymphocytes, the expression of inflammatory cytokines and SLS penetration were assessed, as well.

RESULTS: Treatment with the 10% of glycerol and both concentrations of xylitol inhibited the SLS-induced elevation of TEWL, moderated the irritant-induced increase in dermal blood flow and in the number of leukocyte-endothelial interactions. All concentrations of the applied polyols improved hydration and prevented the accumulation of lymphocytes near the treatment site. At the mRNA level, neither glycerol nor xylitol influenced the expression of interleukin-1 alpha. However, expression of interleukin-1 beta was significantly decreased by the 10% glycerol treatment, while expression of tumor necrosis factor-alpha decreased upon the same treatment, as well as in response to xylitol. Higher polyol treatments decreased the SLS penetration to the deeper layers of the stratum corneum.

CONCLUSION: Both of the analyzed polyols exert considerable anti-irritant and anti-inflammatory properties, but the effective concentration of xylitol is lower than that of glycerol.

Keywords: glycerol; xylitol; sodium lauryl sulfate, acute irritation

Introduction

Irritant contact dermatitis (ICD) is a non-immunologic and non-specific inflammatory disorder of the skin that is caused by physical, mechanical or chemical challenges[1]. Irritation is accompanied by disruption of the barrier and dehydration of the uppermost layer of the skin. Hence, agents contributing to skin hydration and the maintenance of its homeostasis are required for treatment and prevention of ICD. Glycerol, applied in several external formulations, meets these requirements. This polyol exerts anti-irritant effect and acts as a humectant[2, 3]. Moreover, glycerol improves barrier function, hydrates the skin, stabilizes skin collagen and accelerates wound healing[4, 5]. All these raise the question whether structurally similar polyol molecules exhibit similar properties. Xylitol is a naturally occurring polyol, which is a widely used substitute of sugar. Recent studies indicated that xylitol can be utilized as humectant and moisturizer, as well[6, 7]. In our previous study we have shown that both glycerol and xylitol suppresses the 0.1% sodium lauryl sulfate (SLS)-induced acute skin irritation[8]. However, it is not clear whether these polyols are able to provide protection against a more serious irritation. Despite their similar chemical structure, *in vitro* examinations suggest that glycerol and xylitol evoke different gene expression changes in keratinocytes. Glycerol was found to decrease the expression of human leukocyte antigen DR while xylitol increased filaggrin expression[9]. Currently, we have a lot of data on the effects of glycerol on the skin and its potential mechanism of action[5], but much less information is available on the applicability of xylitol.

Our goal was to examine and compare the effects of different concentrations of glycerol and xylitol on skin barrier function, skin hydration, dermal microcirculation, increase our knowledge on the cellular and molecular factors of inflammation and SLS penetration using an animal model of skin irritation.

Materials and Methods

Animals

12-15-week-old male SKH-1 hairless mice were housed in plastic cages in a thermoneutral environment with a 12h light-dark cycle and had access to standard laboratory chow and water *ad libitum*. All interventions were in full accordance with the NIH guidelines and protocols were approved by the Ethical Committee for the Protection of Animals in Scientific Research at the University of Szeged (license number: V./145/2013). Animals were anesthetized with a mixture of ketamine (90 mg/kg body weight) and xylazine (25 mg/kg body weight) administered intraperitoneally. In the end of the experiments, mice were euthanized with an overdose of ketamine (300 mg/kg).

Experimental design (**Fig. 1**)

Mice were randomly allocated into six groups. Group 1 (n=23) served as a control treated with purified water. Group 2 (n=23) was exposed to a 5% solution of SLS. In group 3 (n=23), the solution applied to the skin contained SLS (5%) and glycerol of 5%. The animals of group 4 (n=23) received a solution with SLS (5%) and xylitol of 8.26%. Group 5 (n=23) was treated with SLS (5%) and glycerol of 10%, while in group 6 (n=23), SLS (5%) and xylitol of 16.52% were applied to the skin. SLS and the polyols were dissolved in purified water. Osmolarities of appropriate glycerol and xylitol solutions were equivalent. Groups and treatments are summarized in **Table 1**. In each group, 15 mice were randomly chosen for patch testing followed by attenuated total reflectance Fourier transformed infrared spectroscopy (ATR-FTIR) or determination of transepidermal water loss (TEWL) and skin hydration. After sacrificing the animals, the treated skin was excised and divided into two parts. One part was used to determine either myeloperoxidase (MPO) activity or the expression of inflammatory cytokines (interleukin-1 alpha and beta – IL-1 α and IL-1 β , tumor

necrosis factor-alpha – TNF- α) by means of real time polymerase chain reaction (RT-PCR). Half of the remaining part was fixed in a solution of formaldehyde for routine histology and the other half was subjected to immunofluorescent staining.

Dorsal skin fold chamber was implanted to the dorsal region of the other 8 mice in order to study the microcirculation.

Patch testing

In closed patch tests, extra-large Finn Chambers (diameter of 18 mm) and corresponding filter discs soaked with 120 μ L of the test solutions were applied to the dorsal region for 3 hours using Scanpore tape. The relative humidity was 40-50% and the ambient temperature was kept at 20-22 °C.

Combined tape stripping and ATR-FTIR experiments

For the ATR-FTIR experiments, corneocytes were obtained with adhesive cellophane tape from the patch-tested mice dorsal region. This was repeated up to 25 strips recording an IR spectrum after each third tape strip. Every first tape with one strip was discarded because of the possibility of surface contamination. The untreated dorsal skin of mice was stripped and measured with the same method. ATR-FTIR spectra were recorded with an Avatar 330 FT-IR spectrometer equipped with a horizontal ATR crystal (ZnSe, 45°), between 4000 and 400 cm^{-1} , at an optical resolution of 4 cm^{-1} . 64 scans were co-added and all spectral manipulations were performed by using Thermo Scientific's GRAMS/AI Suite software. In order to obtain a reference spectrum of the API, a KBr pellet containing 0.5 mg SLS was prepared and used. The spectra of the preparations were measured. The spectra of treated and untreated samples were also recorded. Each spectrum of the individual layers of the treated stratum corneum (SC) – containing three strips – was corrected with the spectrum of the own untreated layer. To ensure that no absorbances from the skin itself are remaining and

interfering the results, spectra of untreated control skin samples were also subtracted from spectra of water treated control skin samples. No ATR correction was performed.

Measurement of TEWL and skin hydration

TEWL and hydration measurements were carried out before application of Finn Chambers and 15 min after their removal. TEWL was assessed with a Tewameter TM300 and skin hydration was determined with a Corneometer CM825.

Implantation of dorsal skin fold chamber

Eight mice of each group were implanted by dorsal skin fold chambers according to a previously described method[10]. After a 24-h recovery period, a filter disc with 120 μ L of the test solution was applied to the non-wounded side and covered with a non-permeable film for 3h.

Intravital videomicroscopy

The microcirculation was visualized with a fluorescent intravital videomicroscope (Zeiss). The anesthetized mice received a retrobulbar injection of 2% fluorescein isothiocyanate-labeled dextran and 0.2% rhodamine-6G in order to visualize microcirculation and to stain leukocytes. During examinations, the tissue was superfused with 37°C saline. The microcirculatory parameters were assessed quantitatively off-line by frame-to-frame analysis of the videotaped images. The red blood cell velocity (RBCV, μ m/s) was measured in 4 separate fields of view, in at least 6 capillaries. Leukocyte-endothelial cell interactions were analyzed within 5 postcapillary venules per animal. Adherent leukocytes were defined in each vessel segment as cells that did not move or detach from the endothelial lining within an observation period of 30s.

Routine histology

The hematoxylin-eosin stained 3 µm thick coded sections were analyzed with Panoramic Viewer software. Thickness of viable epidermis (stratum corneum not included) was measured at 20 different points of each section.

Immunofluorescent staining for lymphocytes

The 6 µm cryosections were stained with phycoerythrin conjugated rat-anti mouse CD3 antibody in 1:20 dilution. Nuclear staining with 4', 6-diamidino-2-phenylindole (DAPI, 1:100 dilution, 10 min incubation) was also performed. The sections were examined with an AxioImager.Z1 microscope (Zeiss). Images of the sections were analyzed with ImageJ software. The number of CD3+ cells was referred to 50 basal keratinocytes.

RT-PCR

Total RNA was isolated using RNeasy Mini Kit (Qiagen) according to the manufacturer's instructions. For PCR, amplification was carried out in duplicates of a total volume of 20 µL per sample. Subsequent RT-PCR was performed to quantify transcript abundance using custom primer sets and the Universal Probe Library (Roche) with an iQ Supermix (Bio-Rad) in an LC480 real-time PCR machine (Roche). Relative quantification was carried out using the $2^{-\Delta\Delta CT}$ method and β 2-microglobulin as an endogenous control.

Tissue MPO activity

The activity of MPO, a marker of polymorphonuclear granulocyte accumulation in inflamed tissues, was measured by the method of Kuebler et al.[11]. The MPO activities of the samples were measured at 450 nm (UV-1601 spectrophotometer) and the data were referred to the protein content.

Statistical analysis

Data analysis was performed with SigmaStat for Windows software. Differences among groups were analyzed with Kruskal-Wallis one-way analysis of variance on ranks, followed by Dunn method for pairwise multiple comparison. $P < 0.05$ was considered statistically significant.

Results

TEWL displayed only slight changes in the purified water-treated control group, while exposure to SLS led to a significant elevation in TEWL and application of glycerol of 5% failed to prevent this alteration. Xylitol and glycerol at concentrations of 8.26% and 10%, respectively, improved barrier function. Xylitol of 16.52% restored TEWL values to the control levels after irritant treatment (**Fig. 2**).

In skin hydration, neither purified water in the control group nor SLS alone resulted in a remarkable alteration. However, glycerol and xylitol at both concentrations significantly increased hydration as compared to control and SLS-treated groups (**Fig. 3**).

SLS-induced inflammation led to a considerably faster local blood flow compared to the control animals. 10% glycerol and xylitol at both applied concentrations were found to be able to prevent the irritant-induced RBCV elevation (**Fig. 4.(a)**).

The number of sticking leukocytes detected in the control group was relatively low, while the SLS-treated group was characterized by significantly more leukocyte-endothelial interactions. 5% glycerol was able to partially decrease leukocyte sticking as compared to SLS-treated group. Xylitol, applied at both concentrations, as well as 10% glycerol treatment, effectively prevented leukocyte-endothelial interactions as compared to the control group (**Fig. 4.(b)**).

Epidermal thickness decreased by the exposure to SLS. Both polyols at all applied concentrations increased epidermal thickness considerably as compared to control and SLS-treated groups (**Fig. 5**).

Addition of polyols to SLS prevented the elevation of lymphocyte number induced by the irritant (**Fig. 6**).

Exposure to SLS led to an elevation in the mRNA expression of IL-1 α but neither glycerol nor xylitol interfered with this alteration (data not shown). SLS-induced inflammation was

accompanied by a considerable elevation in IL-1 β mRNA expression level as compared to the control group, which was inhibited only by the 10% glycerol treatment (**Fig. 7.(a)**).

mRNA expression of TNF- α was found to be significantly higher in SLS-treated group compared to control group. Again, 5% glycerol had no effect, whereas 10% glycerol and xylitol applied in both concentrations were effective (**Fig. 7.(b)**).

Irritation with SLS also led to a considerable increase in MPO activity as compared to control group. Addition of the higher concentrations of the polyols to SLS effectively decreased the MPO activities (**Fig. 8**).

ATR-FTIR spectroscopy revealed the most characteristic bond of SLS assigned to SO₂ stretch (asymmetric) at 1220 cm⁻¹ (**Fig. 9**)[12]. It was found that both lower concentration polyol treatments yet modified the SLS penetration, however, significant decrease in the SLS amount in the deeper layers was observed applying the higher polyol treatments (**Table 2**).

Discussion

SLS-induced inflammation is one of the most widely applied models of skin irritation[13], however, concentration of the experimentally administered SLS is a pivotal question[14]. According to previous data, 5% of SLS is sufficient to evoke an inflammation with visible signs and to increase skin blood flow but does not induce epidermal necrosis[15]. In our experiment, 3-hour-exposure to 5% SLS leads to an elevation of TEWL, which may be explained by hyperhydration of the stratum corneum (SC) and possible disorganization of lipid bilayers[16]. On the basis of previous studies, it has been expected that glycerol is able to moderate the irritant-induced TEWL changes[17, 18]. An explanation of the beneficial effects of glycerol may be that it stabilizes the lipids in SC and changes the water binding ability of proteins and lipids[19, 20]. However, increase in water holding capacity of SC reaches a plateau when applied glycerol concentration is higher than 3% after irritant challenge[18].

Our present study has shown that 10% glycerol and the xylitol treatment prevented the TEWL elevation caused by the 5% SLS treatment. The reason why our findings differ from those of other studies may be because of differences in experimental setups. Atrux-Tallau et al. applied a considerably high concentration of SLS (10%), which may have resulted in such severe SC-disruption that lipid-stabilizing effect of glycerol was not sufficient to sustain the barrier function[18]. However, according to our results, barrier damage induced by 5% SLS could be repaired by 10% glycerol. Another important difference is that we applied the polyols together with SLS, while the mentioned other studies used the glycerol after the irritation.

In addition to glycerol, xylitol has been proven to be an effective anti-irritant when applied together with 0.1% of SLS[8]. In the present study, we provide evidence that xylitol can ameliorate a more serious inflammation, as well, and can exert its beneficial effect at a lower concentration compared to glycerol. Although it is difficult to predict the *in vivo* activity of

polyols on the basis of their chemical structure[21], efficacy of xylitol may originate in its water binding capacity.

The results underline the moisturizer effect of both polyols in terms of other parameters, too. SC hydration was found to decrease considerably when the mouse skin was exposed to 10% of SLS[18], however, our results revealed that 5% of SLS did not significantly reduce hydration in mice and both glycerol and xylitol raised hydration values. Moreover, the moisturizing effect of the polyols affected not only the SC, but also the viable epidermis, as it is demonstrated by elevation of epidermal thickness. Beyond skin hydration[22], inflammation-related edema[23] may also lead to a thicker epidermis. In our experiments, increased TEWL levels were detected, which may explain the reduction in epidermal thickness after SLS-treatment. According to our observations, the higher loss of water seems to exceed inflammation-caused edema and the thicker epidermis in polyol-treated groups shows the hydrating effect of glycerol and xylitol.

It is known that exposure to SLS is accompanied by increased dermal blood flow[15, 24]. SLS-induced elevation in RBCV was successfully moderated by 10% glycerol and both applied concentrations of xylitol. Glycerol may directly affect microcirculation: it has been reported that 100% anhydrous glycerol reduces blood flow velocity and vessel diameter of micro-vasculatures[25]. As glycerol we used was one magnitude lower than that in the mentioned study and application of polyols decreased not only dermal blood flow but also other indicators of inflammation, we presume that in this examination, decrease in RBCV in polyol-treated groups seems to originate in the anti-inflammatory activity of these compounds.

MPO is a frequently examined marker in murine models of ICD[26-29]. Our results suggest that SLS-treatment resulted in a considerable accumulation of neutrophil granulocytes, which

was prevented by the application of polyols. Both IVM and MPO activity results confirmed that glycerol and xylitol can influence the neutrophilic activity in this model of irritation.

Although lymphocyte accumulation is more characteristic of atopic or allergic contact dermatitis, irritation may also be accompanied by elevated number of lymphocytes in the skin. According to the available literature data, application of SLS induces T cell-dominated inflammatory response in mouse oral mucosa[30]. In line with these results, our previous study has also demonstrated that exposure to SLS can lead to lymphocyte accumulation in the skin of SKH-1 mice[31]. This study provides evidence that the applied polyols are able to reduce the number of lymphocytes at the irritation site.

However, it is also necessary to characterize the impact of polyols on the cornerstone mediators of inflammatory response. Keratinocytes produce cytokines and chemokines (e.g. IL-1 α , IL-1 β , TNF- α , IL-8) after irritant challenge. The cytokines activate other cell types (e.g. dendritic, Langerhans, endothelial cells) whose cytokine release recruits neutrophil granulocytes, macrophages, mast cells and lymphocytes[32]. TNF- α is of special importance because migration of both dendritic and Langerhans cells require this cytokine and IL-1 β is also essential for the activation of the latter cell type[33, 34]. In murine models, elevation of TNF- α and IL-1 β can be expected in response to SLS treatment[35]. Furthermore, IL-1 α also contributes to the inflammatory response[36]. In this study, both glycerol and xylitol decreased the mRNA expression of IL-1 β and TNF- α , but had no effect on the IL-1 α levels. These may explain their beneficial effects on cellular factors of inflammation, but the exact mechanism via which glycerol and xylitol affect the cytokine expression requires further examinations.

Finally, as glycerol may reduce the average aqueous pore radius in the SC, hereby decreasing the penetration of irritants[37], one may presume that the anti-irritant and anti-inflammatory effects of glycerol and xylitol are just elucidated by the hampered penetration of SLS.

Therefore, the penetration characteristic of SLS in the presence of the polyols, with combined tape stripping and ATR-FTIR spectroscopy[38, 39] was also studied. SLS penetration to the deeper SC layers decreased significantly at higher polyol concentrations, which is a novel feature for xylitol. However, this result is not completely in accordance with the previous observed effects of polyols, where xylitol merely proved to be effective already at lower concentration. In addition, hydrating effects of polyols is independent from their action on SLS penetration. Therefore, mechanism of action of glycerol and xylitol in acute ICD is thought to be partially due to their inhibition on SLS penetration but other unknown mechanisms should also exist.

In conclusion, glycerol and xylitol effectively maintain the barrier function of the skin in SLS-induced acute irritation and are able to hydrate the epidermis and exhibit anti-inflammatory activities. They prevent the inflammation-related increase in dermal blood flow, diminish the accumulation of neutrophil granulocytes and lymphocytes, moderate the expression of inflammatory cytokines and SLS penetration. The effective concentration of xylitol is lower than that of glycerol. According to these results both polyols may contribute to the prevention of ICD.

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Figures

Fig. 1: Experimental setup

Fig. 2: Transepidermal water loss (TEWL) after exposure to purified water (contr.) (white box), sodium lauryl sulfate (SLS) (black box), SLS plus glycerol (G) at 5% (gray box) and 10% (checked gray box) or to SLS plus xylitol (Xy) at 8.26% (striped box) and 16.52% (checked white box). Values are given as percentage referred to TEWL before treatments. The plots demonstrate median with 25th and 75th percentiles. #: p<0.05 vs control, §: p<0.05 vs SLS-treated group.

Fig. 3: Changes in skin surface hydration in purified water-treated (white box), SLS-treated (black box), SLS+5% glycerol-treated (gray box), SLS+8.26% xylitol-treated (striped box), SLS+10% glycerol-treated (checked gray box) and SLS+16.52% xylitol-treated (checked white box) animals. Values are given as percentage referred to hydration before the treatments. The plots demonstrate median with 25th and 75th percentiles. #: p<0.05 vs control, §: p<0.05 vs SLS-treated group.

Fig. 4 4.(a): Red blood cell velocity (RBCV) in dermal capillaries after 3h of treatment in groups 1-6. Values are given in $\mu\text{m/s}$, the plots demonstrate median with 25th and 75th percentiles. #: p<0.05 vs control, §: p<0.05 vs SLS-treated group. **4.(b):** Number of adherent leukocytes in the dermal postcapillary venules after 3h of treatment. Cell numbers are referred to the area of vessel wall and given in $(\text{cell}/\text{mm}^2)*10^2$. The plots demonstrate median with 25th and 75th percentiles. #: p<0.05 vs control, §: p<0.05 vs SLS-treated group.

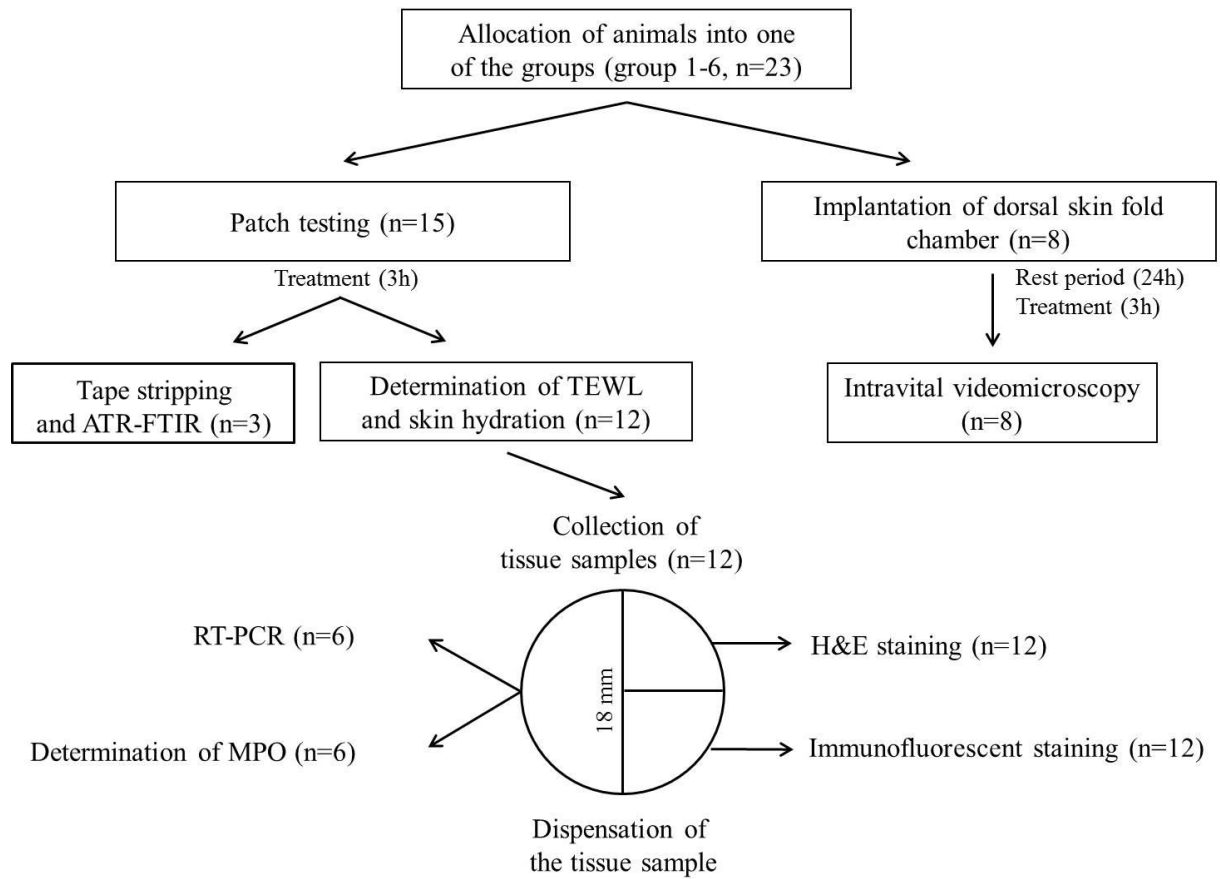
Fig. 5: Photomicrographs of dorsal skin from groups 1-6 (hematoxylin-eosin staining). The graph shows the epidermal thickness in different groups (μm). 50x magnitude, scale bar represents 50 μm . Values are given as median with 25th and 75th percentiles. #: p<0.05 vs control, §: p<0.05 vs SLS-treated group.

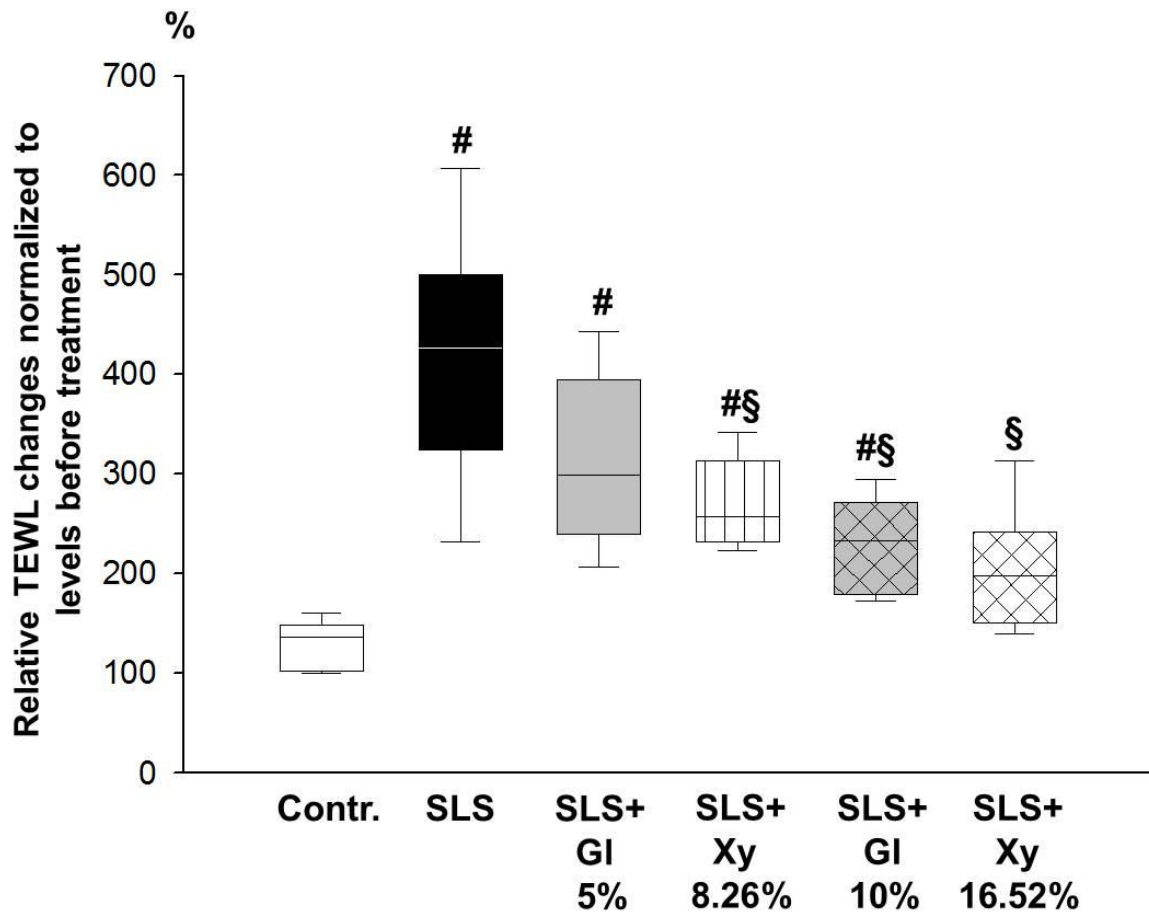
Fig. 6: Presence of lymphocytes in the treated skin in groups 1-6 (DAPI-staining, lymphocytes appear red). The graph demonstrates the number of CD3+ lymphocytes referred to 50 basal keratinocytes. 10x magnitude, scale bar represents 100 μm . Values are given as median with 25th and 75th percentiles. #: $p < 0.05$ vs control, §: $p < 0.05$ vs SLS-treated group.

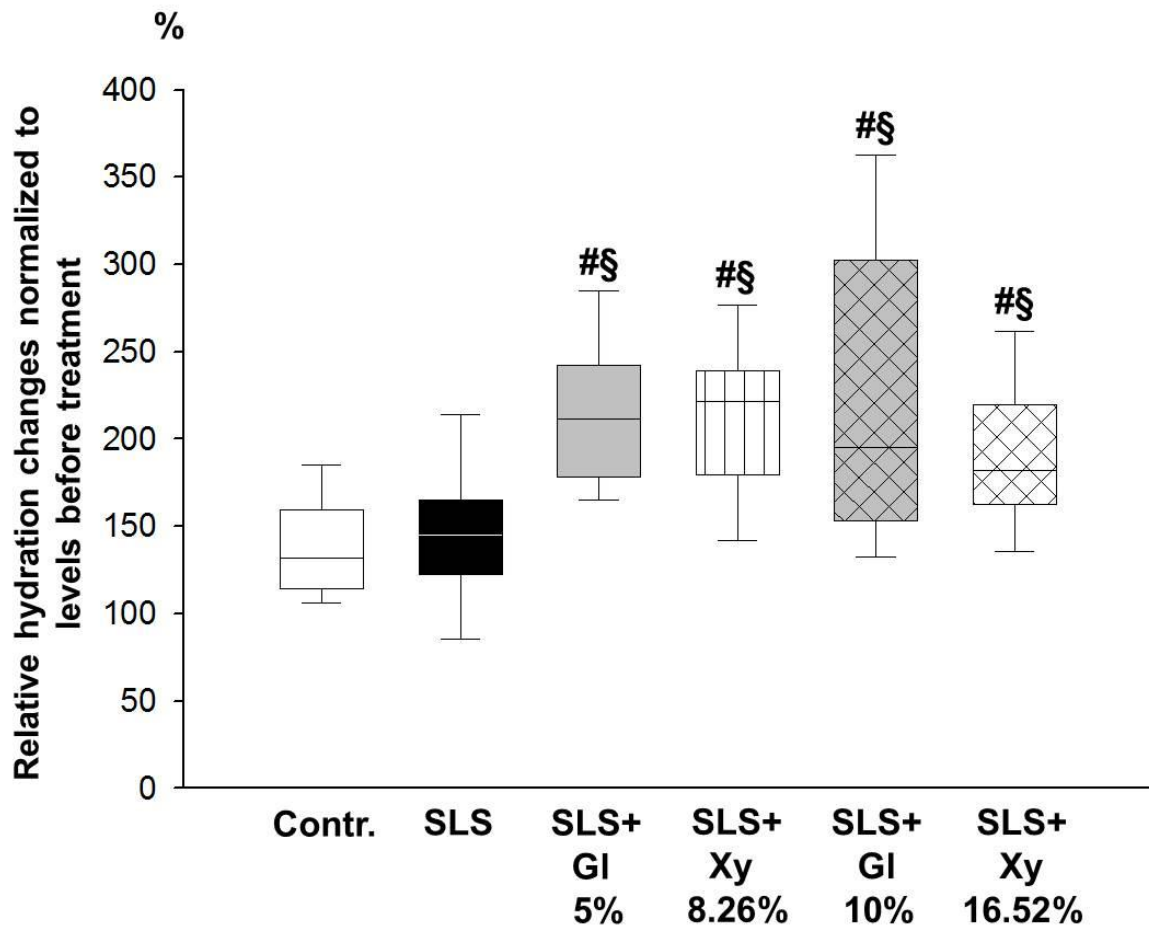
Fig. 7 7.(a): Expression of IL-1 β mRNA after different treatments. Values are given as normalized relative expression. The plots demonstrate median values with 25th and 75th percentiles. #: $p < 0.05$ vs control, §: $p < 0.05$ vs SLS-treated group. **7.(b):** Expression of TNF- α mRNA in groups 1-6. Values are given as normalized relative expression. The plots demonstrate median values with 25th and 75th percentiles. #: $p < 0.05$ vs control, §: $p < 0.05$ vs SLS-treated group.

Fig. 8: Myeloperoxidase (MPO) activity in treated skin of different groups. Enzyme activity is referred to protein content and given as mU/mg protein. The plots demonstrate median values with 25th and 75th percentiles. #: $p < 0.05$ vs control, §: $p < 0.05$ vs SLS-treated group.

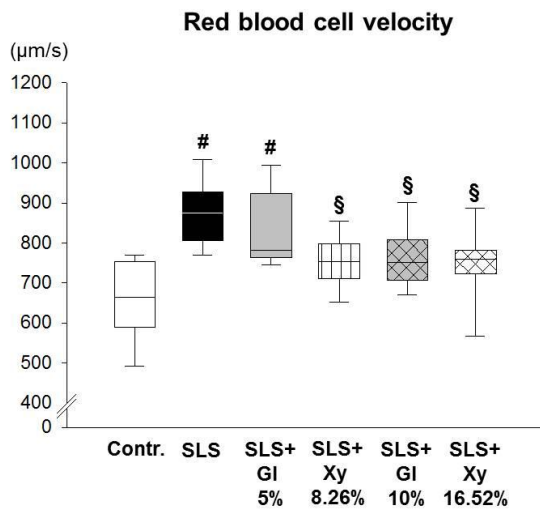
Fig. 9: Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) spectra of the pure SLS, the 5% solution of SLS and the non-treated stratum corneum. Absorbance is given in arbitrary units as a function of the wavenumber.



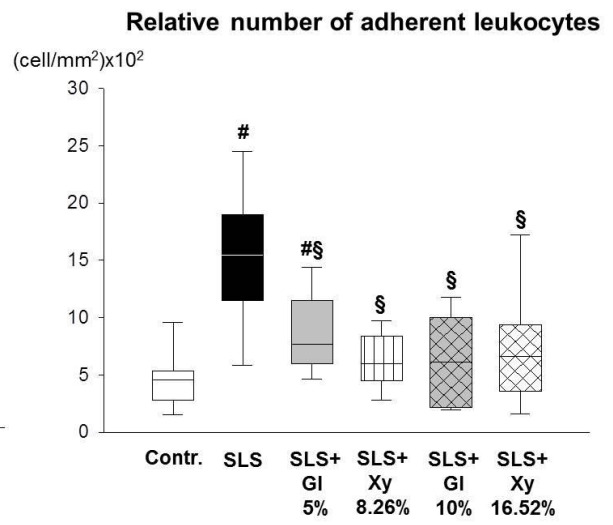


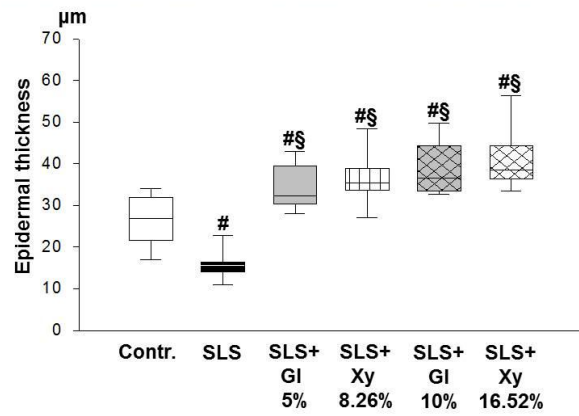
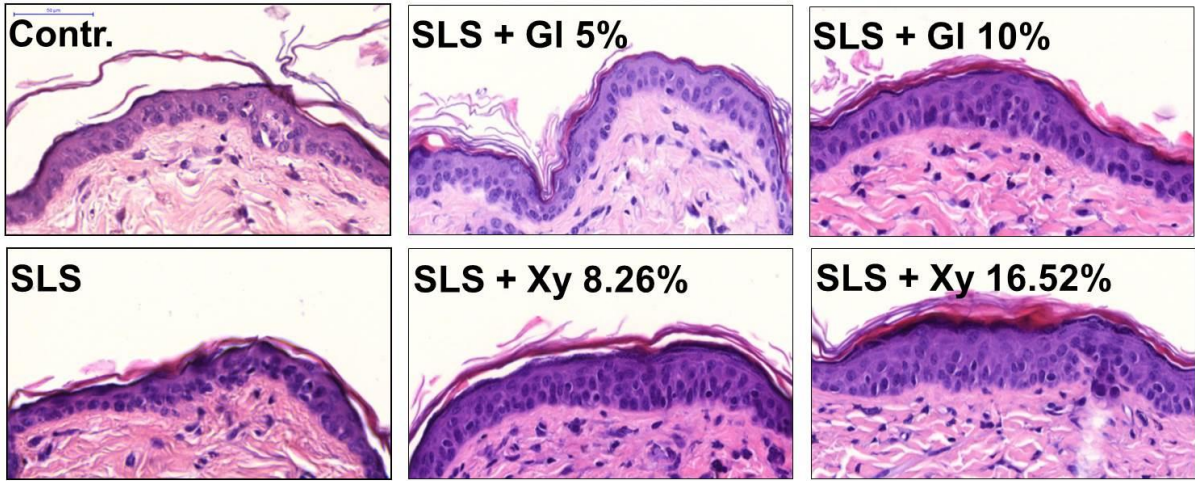


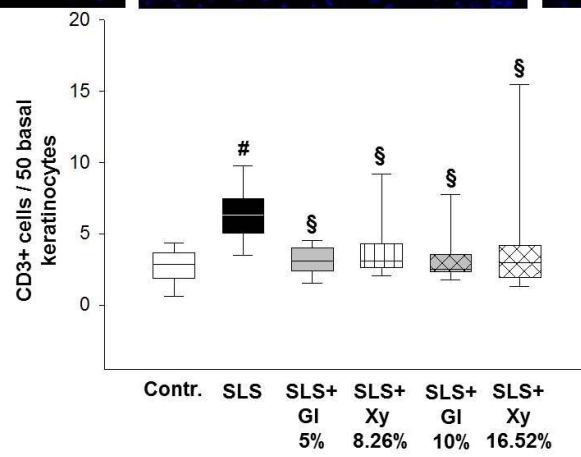
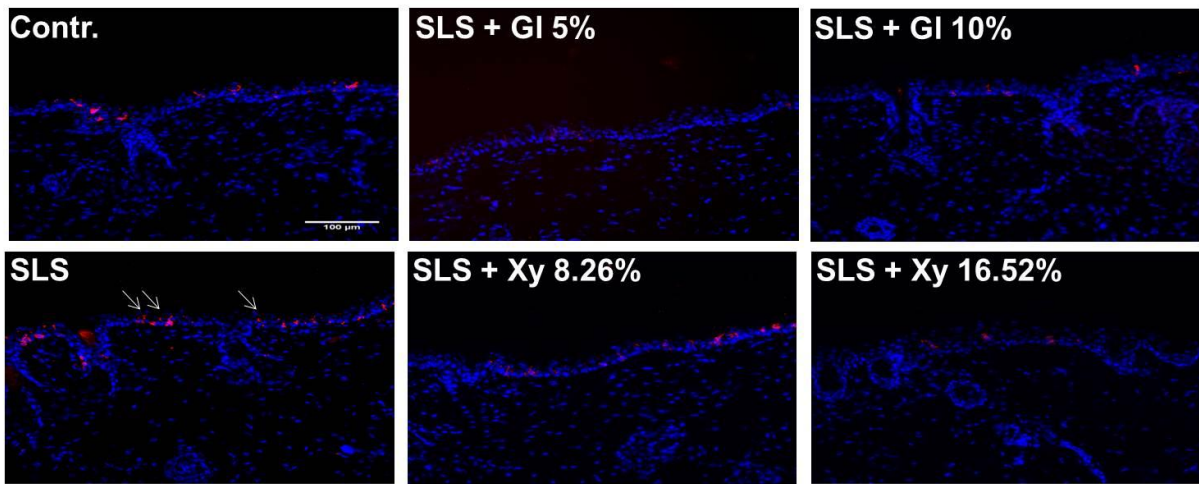
(a)

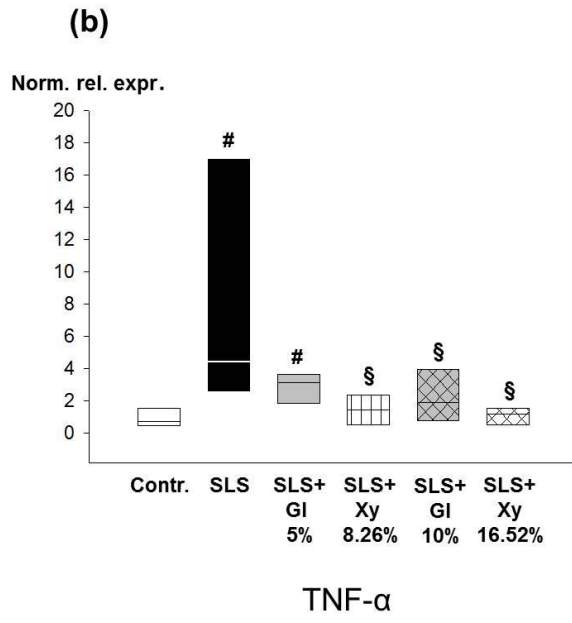
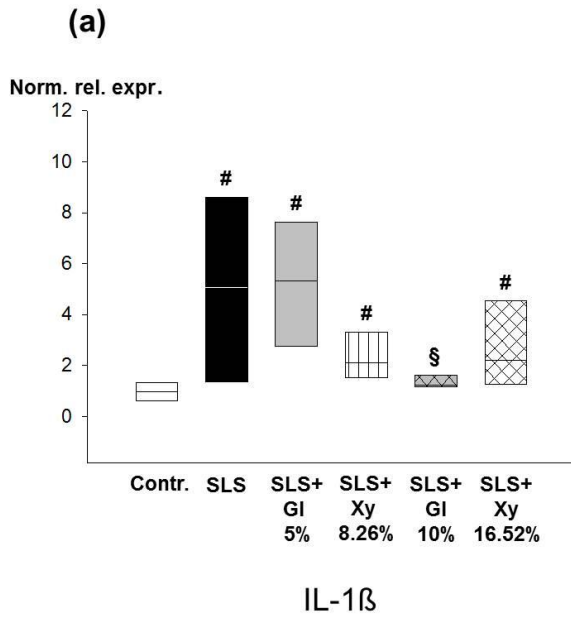


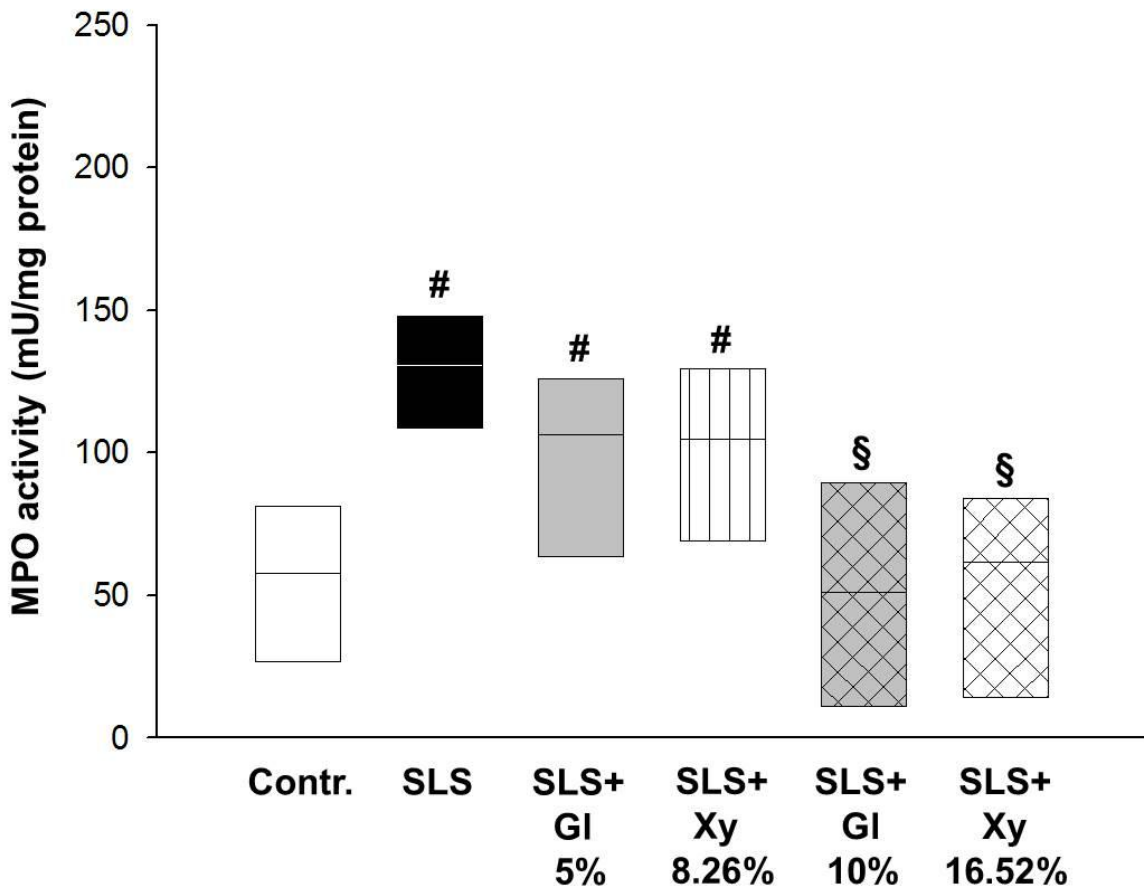
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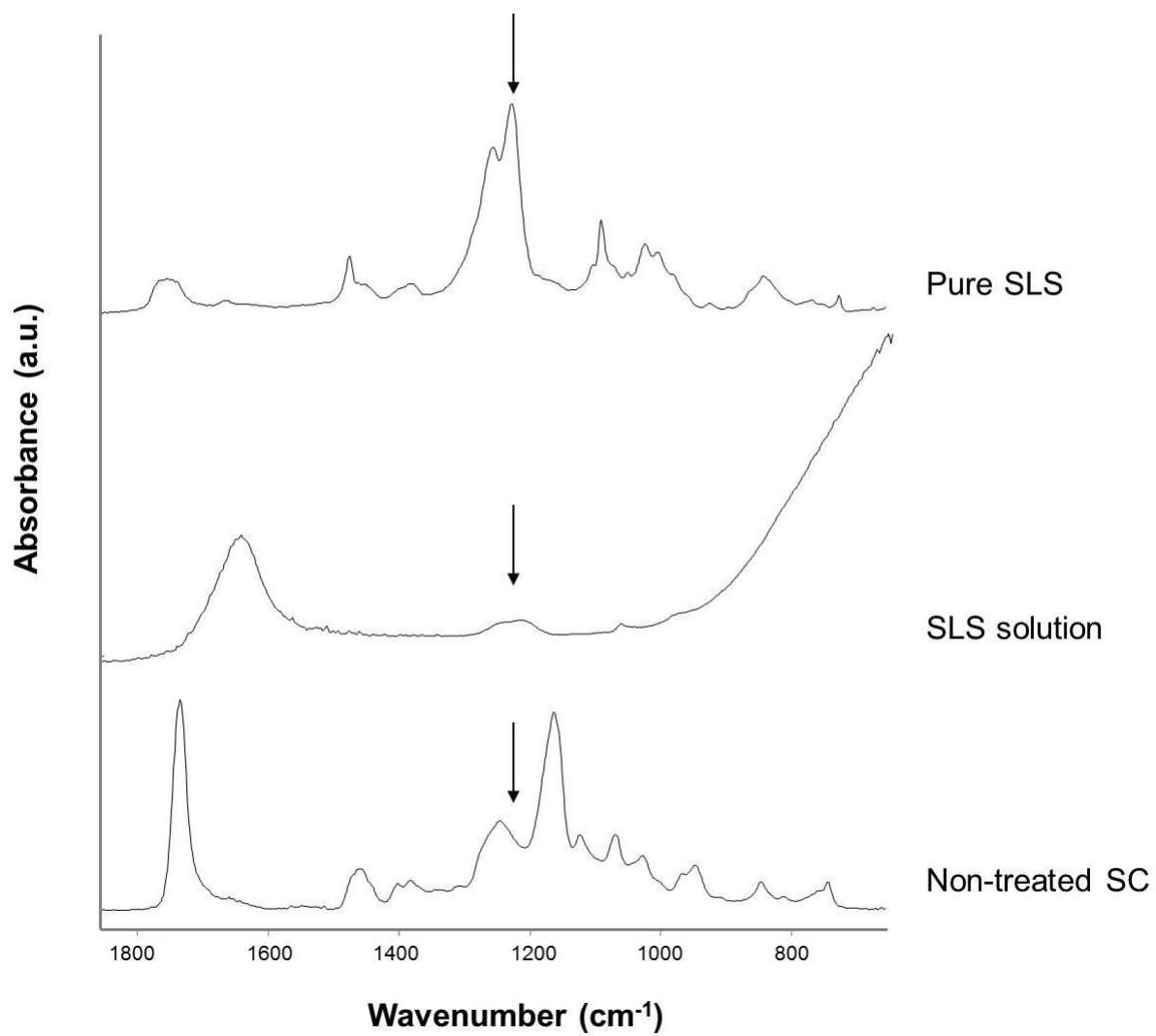












Tables

Group	Applied agents and concentrations
Group 1	Purified water
Group 2	SLS (5%)
Group 3	SLS (5%) + Glycerol (5%)
Group 4	SLS (5%) + Xylitol (8.26%)
Group 5	SLS (5%) + Glycerol (10%)
Group 6	SLS (5%) + Xylitol (16.52%)

Table 1: Study groups, applied agents and their concentration

Number of layers	Group 2 (SLS 5%)		Group 3 (SLS + Glycerol 5%)		Group 4 (SLS + Xylitol 8.26%)		Group 5 (SLS + Glycerol 10%)		Group 6 (SLS + Xylitol 16.52%)	
	M	25p; 75p	M	25p; 75p	M	25p; 75p	M	25p; 75p	M	25p; 75p
1-4	0.0226	0.0137; 0.0264	0.0309	0.0187; 0.0518	0.0318	0.0181; 0.0468	0.0319	0.0161; 0.0532	0.0196	0.0134; 0.0360
5-8	0.0234	0.0140; 0.0433	0.0223	0.0122; 0.0300	0.0241	0.0149; 0.0454	0.00601 *	0.00143; 0.0134	0.000 *	0.000; 0.0133

Table 2 Intensities in the upper and lower layers of the treated stratum corneum (relative absorbance at 1220 cm⁻¹ SLS)

Median values (M) with 25th and 75th percentiles (25p and 75p) of relative absorbance corresponding to the amount of SLS penetrated to the stratum corneum layers (1-4: upper layers, 5-8: lower layers) in the five groups. Group 2 was used as a positive control. * p<0.05 vs. SLS-treated group.