Stratum corneum hydration : mode of action of moisturizers on a molecular level

Caussin, J.

Chapter 4

Lipophilic and hydrophilic moisturizers show different actions on human skin as revealed by cryo scanning electron microscopy

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1. Summary

To study the mode of action of moisturizers on human skin, hydrophilic moisturizers in water and neat lipophilic moisturizers were applied on excised skin for 24h at 32°C. Samples of the treated skin were subsequently visualized in a cryo-scanning electron microscope. The stratum corneum (SC) appeared as a region of swollen corneocytes (the swollen region) sandwiched between two layers of relatively dry corneocytes (the upper and lower non-swelling regions, respectively). Lipophilic moisturizers increased the water content of the SC, whereas hydrophilic moisturizers can also reduce the water content of the SC. When focusing on the effect of the moisturizers on the three different regions, it was observed that cells in the swelling region are most sensitive to the application of the moisturizers and that the change in SC thickness is most influenced by the change in the thickness of the swelling region.

Summarizing, SC cells are not equally sensitive to moisturizer application: centrally located corneocytes are more sensitive than corneocytes in the upper and lowest regions of the SC.

2. Introduction

Dry skin is one of the most common symptoms of dermatological disorders. However, also people with healthy skin may suffer from redness, scaling and itching, which are well-known symptoms of dry skin, due to for instance excessive washing (ElGammal et al., 1996; Rawlings et al., 1994b), or climatic changes (Katagiri et al., 2003a). The aforementioned symptoms are all related to an impaired desquamation process and/or to incomplete maturation of the corneocytes in the SC. A reduction in activity of enzymes involved in these processes can be linked to a lack of water in the SC. Examples of these enzymes are stratum corneum chymotryptic enzyme (SCCE), tryptic enzyme, the cathepsin family and transglutaminases. The former three enzymes play an important role in desquamation (Caubet et al., 2004; Eckert et al., 2005; Egelrud, 2000). Transglutaminases play a crucial role in the maturation of corneocytes (Harding et al., 1999; Hirao, 2003), which has been shown to be very important for the formation of the barrier function of the SC in fresh scars (Kunii et al., 2003). Because of the dependence of the activity of the enzymes on the SC water level, a reduction in this level can disturb the optimal environment for enzymes to maintain an optimal barrier function of the SC. Interestingly, the subsequent imperfect barrier may result in enhanced loss of natural moisturizing factor (NMF) from the SC (Visscher, 2003). Under normal circumstances, this collection of small, hygroscopic substances retains water in the SC. It is produced by yet another water level dependent enzymatic process, the hydrolysis of the SC protein filaggrin (Scott, 1986).

Dry skin is usually treated by the application of moisturizers. Moisturizing treatments have been in use for many years and their ingredients have been studied using several methods. Most of these studies have been performed *in vivo*, using non-invasive techniques, such as TEWL measurements (Rim et al., 2005; Rudolph and Kownatzki, 2004), corneometry (Rudolph and Kownatzki, 2004) or volunteer assessment forms (Holzer et al., 2005; Humbert et al., 2003). While such studies provide valuable information on the effects of moisturizers, they provide almost no information about the working mechanisms of the moisturizers. In order to determine the effect of the treatment on the water distribution in the SC, in the present study the SC of moisturizer-treated skin is visualized using cryo-scanning electron microscopy (cryo-SEM) in combination with cryo-planing (Bouwstra et al., 2003; Nijsse and van Aelst, 1999)

Using this method, the water distribution can be visualized in cross sections of the entire SC, as has been shown previously by Bouwstra *et al* (Bouwstra et al., 2003) for untreated skin and by Richter *et al* (Richter et al., 2004a) for buffer treated skin. In the studies presented here, lipophilic and hydrophilic moisturizers are applied on excised, human skin.

Two groups of moisturizers were used. The first group consists of low molecular weight, hygroscopic substances. It is assumed that, because of their low molecular weight, these substances penetrate the SC, where they subsequently act as humectants (Sagiv and Marcus, 2003), mimicking the role of NMF. However, the molecular weight and their hygroscopic character differs, glycerol being the smallest and most hygroscopic molecule, followed by hydroxyethyl urea (HEU), glucose and saccharose. The second group consists of four lipophilic moisturizers. Lipophilic moisturizers may have at least two working mechanisms. Either they penetrate into the SC and interact with the SC lipids to provide an increased barrier to water loss or they remain on the surface of the skin, thereby preventing evaporation of water from the SC by their occlusive properties. This occlusivity may be caused simply by molecular characteristics, but can also be the result of the formation of organised structures on the surface of the skin (Brinon et al., 1999; Raney and Hope, 2006). The group of lipophilic moisturizers consists of Vaseline (petrolatum) and three isostearic acid derivatives. Vaseline has been speculated to be an occludant, and to increase barrier recovery (Ghadially et al., 1992). The three isostearic acid derivatives have been chosen because of their systemic change in molecular architecture. Isostearyl isostearate (ISIS) is an ester of isostearic acid and isostearic alcohol, while isopropyl isostearate (IPIS) is an ester of isostearic acid and isopropanol. Glycerol monoisostearate (GMIS), is a mono-ester of glycerol and isostearic acid, combining a long, branched carbon chain with a hydrophilic head group.

3. Materials and Methods

Moisturizers

Isostearyl isostearate (ISIS), isopropyl isostearate (IPIS) and glycerol monoisostearate (GMIS) were kindly supplied by Uniqema (Gouda, The Netherlands). Vaseline (petrolatum, VAS, Elida Fabergé, London, United Kingdom) was purchased locally. Glycerol, D-glucose, hydroxyethyl urea and saccharose were purchase from Sigma Aldrich (Zwijndrecht, The Netherlands).

Preparation of skin samples

Human abdomen or mamma skin was obtained after cosmetic surgery and dermatomed to a thickness of approximately 250-300 μm (Padgett Dermatome, Kansas City, KS, USA) the same day, after which it was clamped into Franz type diffusion cells. The acceptor compartment of these cells was filled with PBS pH 7.4 to serve as a source of transepidermal water.

The dermatomed skin was equilibrated in the cells at 32°C over a saturated Na_2CO_3 solution (Bouwstra et al., 2003; Nijsse and van Aelst, 1999) for at least 30 min before a 24h treatment with moisturizers. Hereafter the skin was processed for cryo-planing followed by cryo-scanning electron microscopy. All moisturisers were applied on skin of at least three donors.

Treatments

Skin samples were treated with eight different moisturizers. The lipophilic moisturizers ISIS, IPIS, GMIS and Vaseline were applied neat. The hydrophilic moisturizers GLY, GLU, HEU and SAC were equilibrated at 32°C over a saturated Na_2CO_3 solution, resulting in solutions of 35, 50, 38 and 58% (w/v) moisturizer solutions in water, respectively. At the start of each treatment, these solutions were in equilibrium with the environment, ensuring that the moisturiser concentrations did not change due to evaporation of water. Additionally, an experiment with a 5% glycerol solution was performed to study the effect of a low concentration formulation. For each treatment, either 30 μl/cm^2 lipophilic moisturiser or 50 to 80 μl/cm^2 hydrophilic moisturizer was applied onto the surface of the skin in the donor compartment of the cells. Of each donor, one skin sample was left untreated to serve as a control. After a treatment period of 24 hours over a saturated Na_2CO_3 solution at 32°C, samples of the treated skin and controls were prepared for cryo-SEM.

Cryo scanning electron microscopy

Cryo-planing and cryo-scanning electron microscopy: The method of cryo-planing and cryo-scanning microscopy was described earlier (Bouwstra et al., 2003; Nijsse and van Aelst, 1999; Richter et al., 2004a).

Numerical parameters: For each treatment, skin of three donors (eight donors in total) was used. The minimum number of photographs per treatment per donor was three. In each image, three regions were defined in the upper, central, and lower part of the SC (see figure 1b). The thickness of each three regions was measured manually, and the number of cell layers in each region was counted at two positions in each image. From these data, the average SC thickness, thickness of a region (region thickness), the average number of cell layers in each

region (number of cell layers) and the average thickness of a cell in a region (cell thickness) could be calculated (figure 1b). The averages were compared using unpaired t-tests. Differences with $p<0.05$ were deemed significant (*), differences with $p<0.01$ very significant (**). Because studies using *ex vivo* skin are prone to very large standard deviation, a third term was introduced for differences with $p<0.1$; such differences were referred to as 'trends' (T).

4. Results

SC can be divided into three regions according to their swelling properties

Figure 1a shows a cross section of dermatomed skin equilibrated over a saturated Na_2CO_3 solution at 32°C visualized by cryo-SEM. The image shows a cross section of the SC, the viable epidermis and the upper part of the dermis. In the cryo-planed samples, contrast is created by sublimation of cryofixed free water, which creates holes on the sliced plane. Consequently, areas with only bound water or no water appear as dark, low-contrast areas. In figure 1a, the dermis is visualized as an area of high contrast; long filaments, the collagen fibres, are spread evenly. The viable epidermis is visible as a light grey area with cells and cell nuclei. On top of the viable epidermis, the SC is located. The SC can be divided into three distinct areas: one central area with high contrast sandwiched between two dark areas with low contrast. This indicates that different hydration states exist in different parts of the SC.

The three areas are more clearly visible in figure 1b. Its uppermost region shows no contrast, indicating a lack of free water. It consists of several thin cell layers, and is referred to as the upper non-swelling region. Moving downwards a high-contrast region is visible, indicating the presence of free water inside the corneocytes, located between keratin filaments identified as a white network. Corneocytes are surrounded by the cornified envelope, recognized as a bright boundary. This region is referred to as the swelling region. Below this region, another region is located. Corneocytes in this region contain often small amounts of free water, albeit less than in the swelling region, and is referred to as the lower non-swelling region. This lowest region borders on the epidermis. The three regions described above are observed in nearly all images, although the size of the regions and the number of cell layers in each region may differ between donors, or even between two locations of the same donor. In order to understand the effect of treatment with lipophilic and hydrophilic moisturisers on the inhomogeneous water distribution in the SC, in the present study the three regions are evaluated for their thickness, number of cell layers and average thickness of corneocytes in

each region. First, however, the effect on the thickness of the entire SC is presented, showing whether the SC has or has not retained extra water during moisturizer treatment.

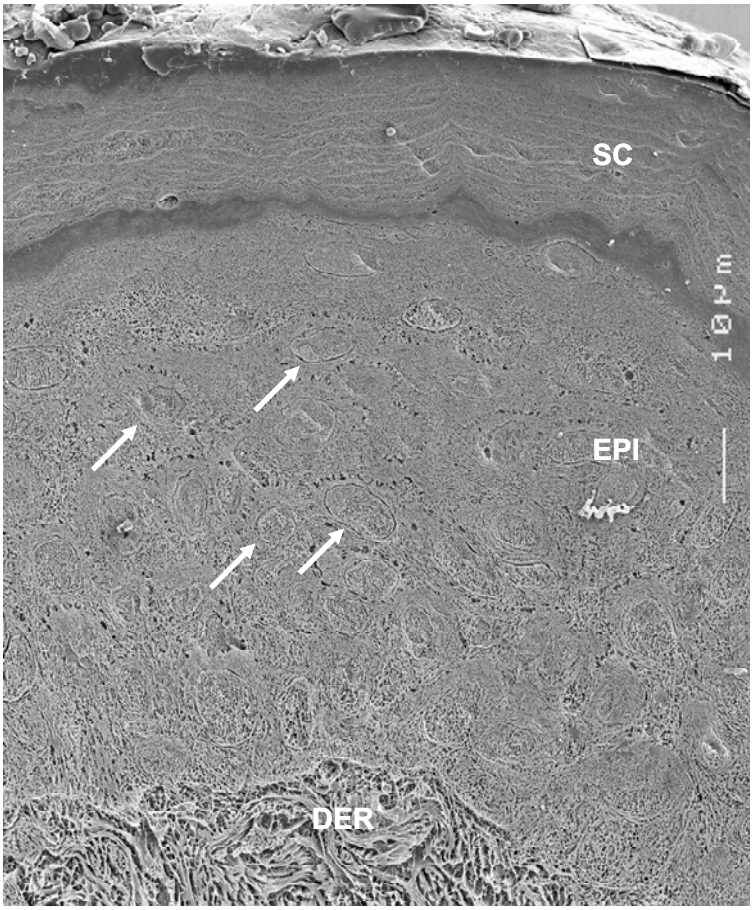

Figure 1a. Cross section of dermatomed skin equilibrated to approx. 80% relative humidity in the skin after cryo-fixation and cryo-planing. The dermis is visualized as an area of high contrast, while the viable epidermis is visible as a light grey area in which cell nuclei (arrows) can easily be recognized. The SC is visible on top of the viable epidermis. Scale bar: 10 µm.

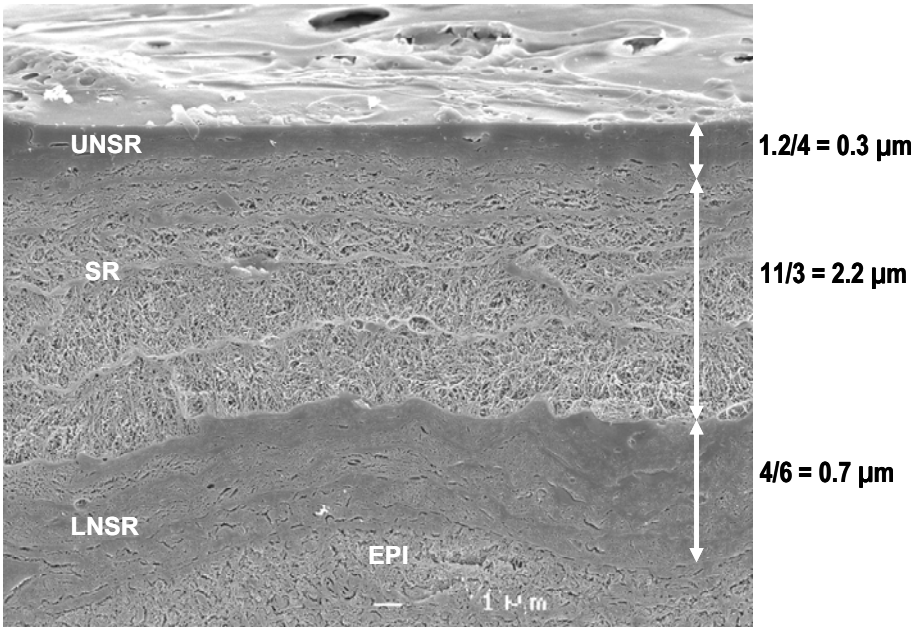

Figure 1b. Cross section of dermatomed skin equilibrated to approx. 80% relative humidity in the skin after cryo-fixation and cryo-planing. The dermis is visualized as an area of high contrast, while the viable epidermis is visible as a light grey area in which cell nuclei (arrows) can easily be recognized. The SC is visible on top of the viable epidermis. Scale bar: 10 µm.

Moisturizer treatment changes total SC water levels only slightly

If moisturizer treatment results in increased water levels in the SC, then its thickness should increase compared to control, as was demonstrated by Bouwstra *et al* (Bouwstra et al., 2003). Figure 2 shows that overall, the thickness of hydrophilic moisturizers treated SC is reduced relative to that of the control, whereas the opposite is observed for SC treated with some lipophilic moisturizers. None of the moisturisers is different from the control, even though there is a trend ($p<0.1$) for glycerol and glucose treated SC to be thinner than the control. There are some significant differences between treatments (table 1A). Vaseline treated SC is significantly thicker than control SC and hydrophilic moisturizer treated SC. ISIS treated SC is thicker than glycerol and glucose-treated SC.

Corneocytes in the swelling region are most affected by moisturizer treatment

From the visual inspection of the micrographs, it is clear that the water distribution in SC is not homogeneous over the depth of the SC (figure 1a and 1b). Figure 1c shows exerts of micrographs of the SC of skin samples treated with the tested moisturisers. The swelling region makes up the greatest fraction of the SC, also after treatment with glycerol and glucose, the moisturisers which appear to decrease rather than increase the total thickness of the SC. The average region thicknesses of the control and after treatment with the hydrophilic and lipophilic moisturizers are shown in figure 2. It is clearly visible that in treated and untreated skin the swelling region is the largest region in the SC. Changes in the upper non-swelling region and lower non-swelling region are so small that they have practically no bearing on the changes in total SC thickness. The swelling region remains the region in which the largest differences in swelling after moisturizer treatment are observed. After treatment with lipophilic moisturizers, the swelling region appears to be up to twice as thick as after treatment with hydrophilic moisturizers. A great number of trends and differences between moisturizer treatments have been observed, mostly between hydrophilic and lipophilic moisturizers (table 1B).

A change in the thickness of a SC region due to the application of a moisturizer may have two reasons. Firstly, it is possible that cells have changed characteristics and are now assigned to another region, for instance by starting to swell. A second possibility is that the water level in a corneocyte increases or decreases, but not to such an extent that it is assigned to another region. For instance, the cell thickness of a swelling region corneocyte may increase even more. Naturally, a combination of both described phenomena is also possible. To determine if

either one or both of these situations is present after the application of lipophilic or hydrophilic moisturizers, the number of cell layers in each region was counted. In combination with the measured thickness of the three regions, the average thickness of corneocytes in the regions was calculated. The results of these calculations are presented below.

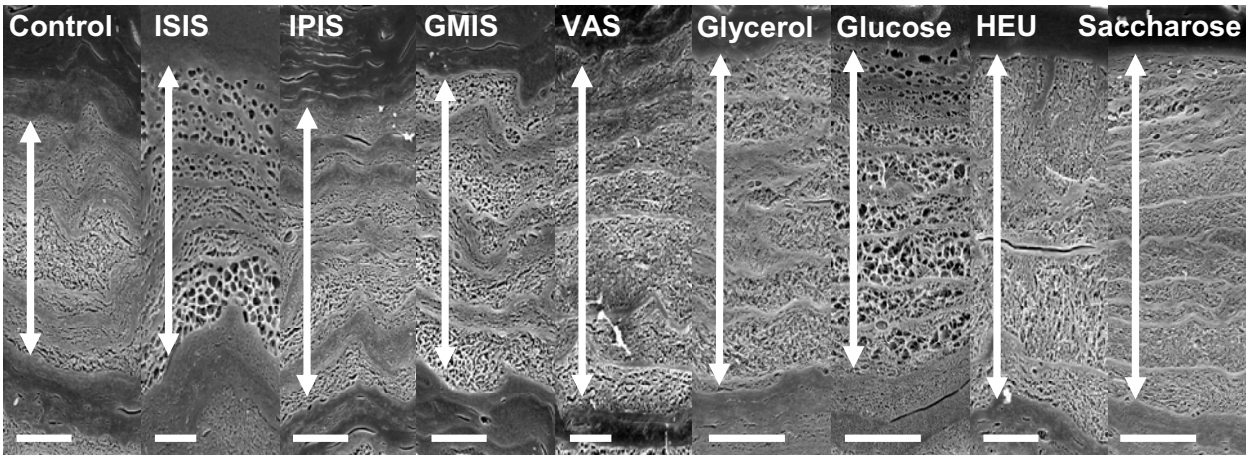

FIGURE 1C. SC cross sections of skin treated with the various moisturisers. The three regions are clearly visible in each cross section. The swelling region is indicated by a two-pointed arrow. Scale bar: 2 µm.

Individual cells or cell layers may change their swelling characteristics during moisturizer treatment

Several trends and differences in the number of cells per region between samples treated with different moisturizers were observed (figure 3, table 1C). Most differences are observed in the upper non-swelling region, and are not only observed between hydrophilic and lipophilic moisturizers, but also between individual moisturizers within the group of hydrophilic moisturizers. More specifically, glycerol and HEU appear to be different from the two sugars glucose and SAC, the latter two having more cell layers in the upper non-swelling region. No differences were observed in the swelling region.

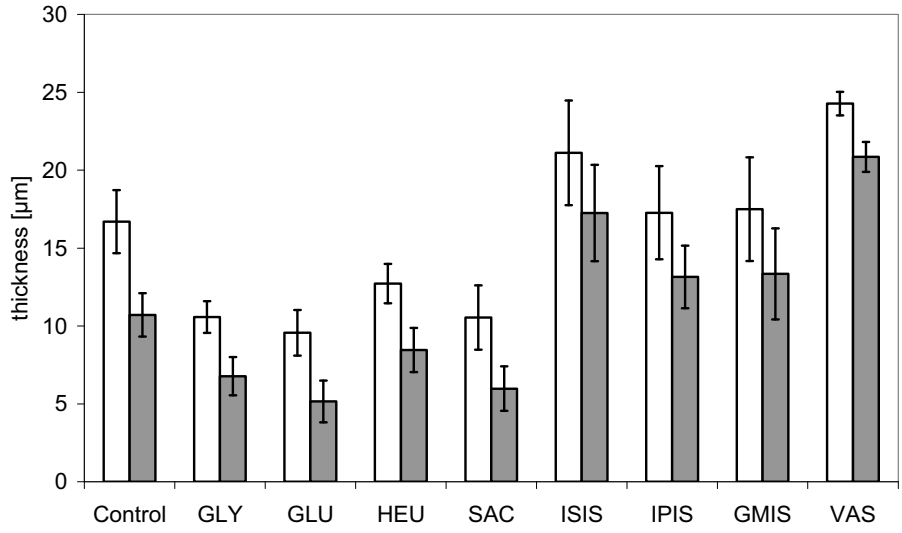

FIGURE 2. Average thickness SEM of SC of untreated and moisturizer treated skin and of its swelling region (filled bar). On average, the thickness of hydrophilic moisturizer treated SC appears smaller than that of the control, whereas the opposite is observed for lipophilic moisturizers treated SC. Only Vaseline (VAS) is significantly thicker than control ($p<0.05$). The swelling region is the largest region in the SC and it is also the region with the largest differences in swelling after moisturizer treatment.

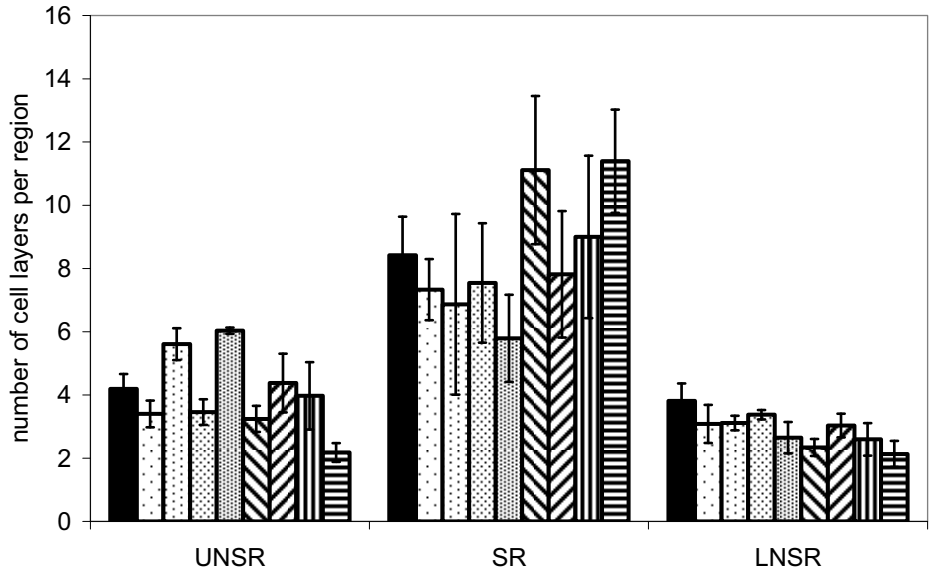

FIGURE 3. Average number of cell layers SEM of the three SC regions of untreated and moisturizer treated skin. The highest number of cell layers is observed in the swelling region (6 to 10 layers), where the hydrophilic moisturizers appear to have fewer layers than the control. The upper non-swelling region has 2 to 6 cell layers and the lower non-swelling region 2 to 3.

Table 1A SC thickness

Trends and significant differences after treatment with different, individual moisturizers. T=p<0.1, *=p<0.05, **=p<0.01

	Control	Glycerol	Glucose	HEU	Saccharose
Control		T	T		
ISIS		*	*	T	T
IPIS			T		
GMIS			T		
Vaseline	*	**	**	**	**

NB There is also a trend for IPIS and Vaseline to be different.

Table 1B Average region thickness in the swelling region

Trends and significant differences after treatment with different, individual moisturizers. T=p<0.1, *=p<0.05, **=p<0.01

	Control	Glycerol	Glucose	HEU	Saccharose
Control			*		
ISIS		*	*	T	*
IPIS		T	*		*
GMIS			T		T
Vaseline	**	**	**	**	T

NB There is also a difference between Vaseline and IPIS (*) and a trend for Vaseline and GMIS to be different.

Table 1C Average number of cell layers in the upper non-swelling region

Trends and significant differences after treatment with different, individual moisturizers. T=p<0.1, *=p<0.05, **=p<0.01

	Control	Glycerol	Glucose	HEU	Saccharose
Control					*
ISIS			*		**
IPIS					
GMIS					
Vaseline	*	T	*	T	**

NB There are also differences between glycerol and glucose (*), glycerol and saccharose (**), glucose and HEU (*) and HEU and saccharose (**)

Table 1D Average cell thickness in the swelling region

Trends and significant differences after treatment with different, individual moisturizers. T=p<0.1, *=p<0.05, **=p<0.01

	Control	Glycerol	Glucose	HEU	Saccharose
Control			T		
ISIS		*	**	T	*
IPIS					
GMIS		T	*		
Vaseline	T	*	*	*	

Is the extent to which corneocytes in a region swell affected by moisturizer treatment?

The differences in average cell thickness between the three regions of the control and after treatment with individual moisturizers are shown in figure 4 and table 1D. Here it can be seen that cells from the swelling region can be 2 to 4 times as thick as the approx. 0.5 µm thick cells from the upper non-swelling region. Cells from the lower non-swelling region, which under normal circumstances already contain some water, may swell to twice the thickness of the very dry corneocytes in the upper non-swelling region. There are also several differences between individual lipophilic and hydrophilic moisturizers in the swelling region, mainly between the hydrophilic moisturizers, Vaseline and ISIS, respectively. In the lower non-swelling region, there are some trends and differences between individual moisturizer-treated skin, not only between the groups, but also within them. Vaseline treated corneocytes are thicker than glycerol-, saccharose- or IPIS-treated corneocytes, whereas GMIS-treated corneocytes are different from those treated with glycerol.

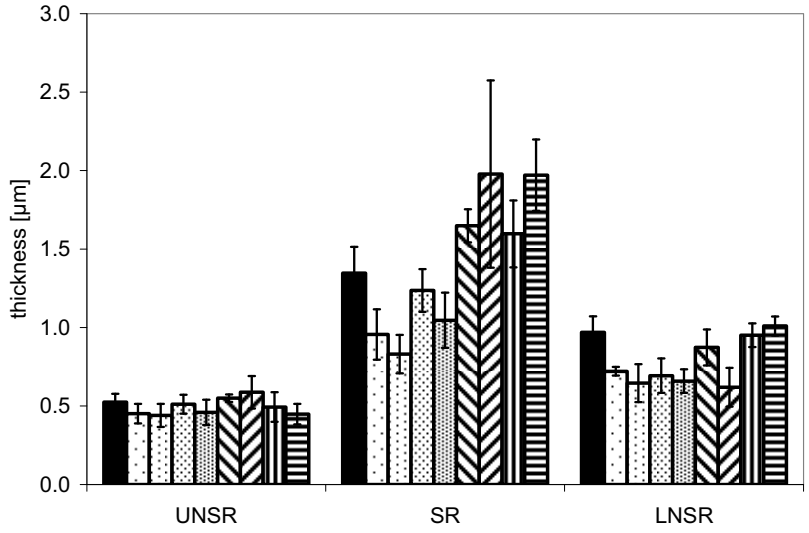

Figure 4. Average cell thickness SEM in the three SC regions of untreated and moisturizer treated skin. Cells from the swelling region can be 2 to 4 times as thick as the approx. 0.5 µm thick cells from the upper non-swelling region.

Low concentrations of hydrophilic moisturizers increase total SC water levels

Figure 5 shows the results of a study performed to study the effect of glycerol concentration on the SC swelling behaviour. This is of interest, as a previous study showed that glycerol at concentrations between 1 and 10% increase murine SC water levels, whereas concentrations above 10% do not (Fluhr et al., 2003). In our study treatment of full skin with 5% glycerol resulted in a 25% increase of total SC thickness compared to the untreated situation.

Determination of the number of cell layers in the three regions as well as the average cell thickness within the regions showed that the increase was caused by an increase in the number of cell layers in the central swelling region, as well as by an increase in the average cell thickness in this region (not shown).

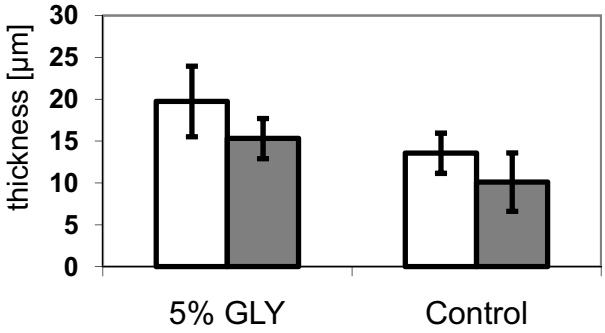

FIGURE 5. Average thickness SEM of SC of 5% glycerol treated and control skin and of its swelling region (filled bar). The increase in total thickness ($p<0.001$) is primarily due to an increase of the size of the swelling region ($p<0.001$).

5. DISCUSSION

In order to effectively treat dry skin, the water content of the SC must be increased to a level at which the enzymes responsible for NMF production, corneocyte maturation and desquamation are fully active. To study the effect of lipophilic and hydrophilic moisturizers on water content, the SC of treated skin was visualized using cryo-SEM. The characteristics of the SC cross sections were examined numerically, and although great variations were found, most likely due to the use of donor skin, some important observations were made. Most importantly, the resulting micrographs of SC could roughly be divided into a swelling region sandwiched between two lower-contrast regions with thinner corneocytes (the upper and lower non-swelling region) (Bouwstra et al., 2003; Richter et al., 2004b). This inhomogeneous pattern of the SC may be explained by the changes in NMF production that corneocytes undergo in the SC. In the layers closest to the epidermis, NMF production has not started yet and water can therefore not be retained. This is most likely the reason why corneocytes in the deepest region, the lower non-swelling region, do not swell much compared to those in the swelling region. In the central layers of the SC, the swelling region, cells swell massively, presumably because much NMF is available for water retention. Moving upwards, the upper non-swelling region is reached. In this region NMF is easily lost due to the skin surface's frequent contact with water (Visscher, 2003; Wiechers, 2003). Therefore the upper corneocytes contain only bound water and appear as dark, unswollen cells, even at 60-90% relative humidity. It would appear that the water distribution observed in this paper excellently correlates with the NMF distribution in SC. The inhomogeneous distribution of NMF described

above has been previously reported (Hashimotokumasaka et al., 1991) and was also shown by Bouwstra *et al* (unpublished). It may be suggested that the observation of a lower non-swelling region is an experimental artefact due to the used *ex vivo* set-up, in which there is no direct contact between the dermatomed skin and a source of water. However, this is clearly in disagreement with the fact that the epidermis and dermis underneath this region appear to contain large amounts of bulk water, as can be deducted from the contrast in the micrographs. Therefore, in principle, sufficient water is present to hydrate the lowest layers of the SC.

The most important question addressed in this paper is whether moisturizer application results in increased water levels in the SC and/or change the water distribution in the SC. The results show that in general the thickness of lipophilic moisturizer treated SC is larger than that of SC treated with hydrophilic moisturizers. However, there are also differences within the hydrophilic and lipophilic moisturizer groups. In the lipophilic group, treatment with ISIS and Vaseline appears to result in the largest increase in SC thickness. This is in agreement with results from earlier NIR studies[1], which have shown them to be better moisturizers than IPIS and GMIS, which are not different from the control. On the other hand it is in disagreement with corneometry results that showed IPIS to be more effective that Vaseline and ISIS (Wiechers, 1999). However, corneometry provides an integral measure over the whole depth of the SC and should result in lower values if the outermost layer acts as an isolating layer, e.g. after application of a moisturizer. Whether or not this might be an explanation of the obtained difference will certainly be subject of future studies. Interestingly, previous results have also shown them to be better moisturisers than IPIS and GMIS (Wiechers, 1999), which are not different from the control. The results of the Vaseline treatment are not surprising, as this is a known occlusive agent; however, ISIS has been shown not to be occlusive (Wiechers J.W., 1999), and must therefore have a different mode of action. It is possible that this moisturizer interacts with the SC lipids and increases their barrier function, a hypothesis which merits further investigations.

In contrast to the results of the lipophilic moisturizers, the application of the hydrophilic moisturizers results in decreased SC thicknesses. This means that after treatment water levels in the SC are lower, which is most likely caused by their relatively high concentrations used in this study to ensure that the formulations are in equilibrium with the relative humidity in the environment at the start of the experiment. It has been shown in mice that glycerol concentrations above 10% do not increase SC levels compared to concentrations between 1

and 10%, which do increase water levels (Fluhr et al., 2003). We observed that concentrations used in this study can also result in a decrease of SC water levels. However, lowering the glycerol concentration to 5% w/w results in an increase of the water level, in accordance to the previous studies (Fluhr et al., 2003). This proposes an interesting difference between the working mechanisms of lipophilic and hydrophilic moisturizers. Lipophilic moisturizers are likely to act by preventing water from evaporating from the SC. Higher concentrations are therefore unlikely to result in effects opposite to moisturization. As such, traditional occlusive moisturisers such as Vaseline could even result in SC water levels too high for SC enzymes involved in e.g. desquamation to work properly and may even disrupt the SC lipid organization. Hydrophilic moisturizers, however, could potentially be used to adjust the SC to the specific water levels necessary for these enzymatic processes by carefully selecting their concentration.

Not surprisingly, the corneocytes in the swelling region are most affected by the moisturizer treatments. The NMF produced in this region enables the corneocyte to retain water and therefore to swell. However, differences between moisturizers in the number of cells in each region suggest that in some cases corneocytes can 'move' from one region to another. Treatment with glucose and SAC in the high concentration used in this study results in an increase in the number of cells in the upper non-swelling region, seemingly dehydrating the SC, rather than moisturising it. These two moisturizers, which caused a decrease in thickness, appear to reduce the water content of corneocytes in the uppermost layers of the swelling region most easily, most probably because these layers have less NMF than the central or lower swelling region corneocytes. This reduces the osmolarity in the corneocytes, promoting the partitioning of water from it to its environment. Interestingly, the opposite is visible after treatment with 5% glycerol, where the number of swelling region cells increases (not shown).

Changes in thickness and number of cells in the upper and lower non-swelling region a more difficult to determine, because of the small size of these areas. However, some suggestions can be made. While in the swelling region the number of cells increases after ISIS and Vaseline treatment, the number of cells in the upper non-swelling region decrease. This suggests that non-swelling cells have swollen upon moisturizer treatment. Possibly, the upper non-swelling region cells start to swell when the swelling region corneocytes have reached the maximum of their swelling capacity. This is accompanied by the trend for lower non-swelling region corneocytes, which may be affected in a similar way, to be more swollen after application of lipophilic moisturizers than after application of hydrophilic moisturizers.

In a previous study (Bouwstra et al., 2003), a linear relationship was obtained between the average cell thickness in SC and the SC hydration level. This relationship can used to hypothesize on the water levels in the three regions. The thin cells in the upper non-swelling region and the lower non-swelling region of the control are estimated to contain approx. 50% (w/w) water, whereas corneocytes in the swelling region contain approx 120% of their own weight in water. Treatment with lipophilic moisturizers or low concentrations of glycerol may increase this level in the swelling region to up to 300%, whereas treatment with high concentrations of hydrophilic moisturizers resulted in a hydration of 70-90%, i.e. slightly lower than that of the control. This difference supports the different working mechanisms proposed for the two moisturizer groups, according to which lipophilic moisturizers increase the water content of the SC, and hydrophilic moisturizers regulate it, depending on the moisturizer's concentration in the formulation.

6. Conclusion

This study shows that treatment with moisturisers can change not only the water levels in the SC, but also the location of water in it. We propose that hydrophilic and lipophilic moisturizers affect the SC hydration differently as a result of their different mechanisms of action. Previous work has shown that three of the studied lipophilic moisturisers are not occlusive in the classical sense of the word (Wiechers, 2003). Whether they act by interaction with the intercellular SC lipids, creating an 'internal' occlusive effect will be subject of further studies.

The effect of hydrophilic moisturizers appears to be largely dependent on the concentration of the moisturizer in the treatment formulation. High concentration of the moisturizer in the formulation can decrease the water levels in the SC, contrasting the effect of e.g. glycerol at lower concentrations (Fluhr et al., 2003), which increase water levels. This suggests that hydrophilic moisturisers can be used to regulate SC hydration, rather than only increase it. This is of interest, as the enzymes whose activity is impaired in dry skin also do not function in SC with too high water levels.

7. Acknowledgements

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8. References

Bouwstra, J. A., de Graaff, A., Gooris, G. S., Nijsse, J., Wiechers, J. W., and van Aelst, A. C. (2003): Water distribution and related morphology in human stratum corneum at different hydration levels. *Journal of Investigative Dermatology* 120(5), 750-8.

Brinon, L., Geiger, S., Alard, V., Doucet, J., Tranchant, J. F., and Couarraze, G. (1999): Percutaneous absorption of sunscreens from liquid crystalline phases. *Journal of Controlled Release* 60(1), 67-76.

Caubet, C., Jonca, N., Brattsand, M., Guerrin, M., Bernard, D., Schmidt, R., Egelrud, T., Simon, M., and Serre, G. (2004): Degradation of corneodesmosome proteins by two serine proteases of the kallikrein family, SCTE/KLK5/hK5 and SCCE/KLK7/hK7. *Journal of Investigative Dermatology* 122(5), 1235-1244.

Eckert, R., Sturniolo, M., Broome, A., Ruse, M., and Rorke, E. (2005): Transglutaminase function in epidermis. *Journal of Investigative Dermatology* 124(3), 481-492.

Egelrud, T. (2000): Desquamation in the stratum corneum. *Acta Dermato-Venereologica*, 44-45.

ElGammal, C., Pagnoni, A., Kligman, A. M., and ElGammal, S. (1996): A model to assess the efficacy of moisturizers - The quantification of soap-induced xerosis by image analysis of adhesive-coated discs (D-Squames(R)). *Clinical and Experimental Dermatology* 21(5), 338-343.

Fluhr, J. W., Mao-Qiang, M., Brown, B. E., Wertz, P. W., Crumrine, D., Sundberg, J. P., Feingold, K. R., and Elias, P. M. (2003): Glycerol regulates stratum corneum hydration in sebaceous gland deficient (Asebia) mice. *Journal of Investigative Dermatology* 120(5), 728-737.

Ghadially, R., Halkiersorensen, L., and Elias, P. M. (1992): Effects of Petrolatum on Stratum-Corneum Structure and Function. *Journal of the American Academy of Dermatology* 26(3), 387-396.

Harding, C., Long, S., Rogers, J., Banks, J., Zhang, Z., and Bush, A. (1999): The cornified cell envelope: An important marker of stratum corneum maturation in healthy and dry skin. *Journal of Investigative Dermatology* 112(4), 573-573.

Hashimotokumasaka, K., Horii, I., and Tagami, H. (1991): Invitro Comparison of Water-Holding Capacity of the Superficial and Deeper Layers of the Stratum-Corneum. *Archives of Dermatological Research* 283(5), 342-346.

Hirao, T. (2003): Involvement of transglutaminase in ex vivo maturation of cornified envelopes in the stratum corneum. *International Journal of Cosmetic Science* 25, 245-257.

Holzer, G., Riegler, E., Honigsmann, H., and Schmidt, J. (2005): Effects and side effects of 2%-progesterone cream on the skin of peri- and postmenopausal women: Results from a double blind, vehicle-controlled, randomized study. *Experimental Dermatology* 14(2), 156-157.

Humbert, P. G., Haftek, M., Creidi, P., Lapiere, C., Nusgens, B., Richard, A., Schmitt, D., Rougier, A., and Zahouani, H. (2003): Topical ascorbic acid on photoaged skin. Clinical, topographical and ultrastructural evaluation: double-blind study vs. placebo. *Experimental Dermatology* 12(3), 237-244.

Katagiri, C., Sato, J., Nomura, J., and Denda, M. (2003): Changes in environmental humidity affect the water-holding property of the stratum corneum and its free amino acid content, and the expression of filaggrin in the epidermis of hairless mice. *Journal of Dermatological Science*31(1), 29-35.

Kunii, T., Hirao, T., Kikuchi, K., and Tagami, H. (2003): Stratum corneum lipid profile and maturation pattern of corneocytes in the outermost layer of fresh scars: the presence of immature corneocytes plays a much more important role in the barrier dysfunction than do changes in intercellular lipids. *British Journal of Dermatology* 149(4), 749-56.

Nijsse, J., and van Aelst, A. C. (1999): Cryo-planing for cryo-scanning electron microscopy. *Scanning* 21(6), 372-378.

Raney, S. G., and Hope, M. J. (2006): The effect of bilayer and hexagonal H-II phase lipid films on transepidermal water loss. *Experimental Dermatology* 15(7), 493-500.

Rawlings, A. V., Watkinson, A., Rogers, J., Mayo, A. M., Hope, J., and Scott, I. R. (1994): Abnormalities In Stratum-Corneum Structure, Lipid-Composition, And Desmosome Degradation In Soap-Induced Winter Xerosis. *Journal of the Society of Cosmetic Chemistry* 45(4), 203-220.

Richter, T., Peuckert, C., Sattler, M., Koenig, K., Riemann, I., Hintze, U., Wittern, K. P., Wiesendanger, R., and Wepf, R. (2004a): Dead but highly dynamic - The stratum corneum is divided into three hydration zones. *Skin Pharmacology & Physiology* 17(5), 246-257.

Richter, T., Peuckert, C., Sattler, M., Koenig, K., Riemann, I., Hintze, U., Wittern, K. P., Wiesendanger, R., and Wepf, R. (2004b): Dead but highly dynamic - The stratum corneum is divided into three hydration zones. *Skin Pharmacology &Physiology* 17(5), 246-257.

Rim, J. H., Jo, S. J., Park, J. Y., Park, B. D., and Youn, J. I. (2005): Electrical measurement of moisturizing effect on skin hydration and barrier function in psoriasis patients. *Clinical and Experimental Dermatology* 30(4), 409-413.

Rudolph, R., and Kownatzki, E. (2004): Corneometric, sebumetric and TEWL measurements following the cleaning of atopic skin with a urea emulsion versus a detergent cleanser. *Contact Dermatitis* 50(6), 354-358.

Sagiv, A. E., and Marcus, Y. (2003): The connection between in vitro water uptake and in vivo skin moisturization. *Skin Research Technology* 9(4), 306-311.

Scott, I. H., CR. (1986): Filaggrin proteolysis and the control of stratum corneum hydration. *Journal of Invetsigative Dermatology* 87(1), 167.

Visscher, M. O., Tolia, G.T., Wickett, R.R. , Hoath, S.B. . (2003): Effect of soaking and natural moisturizing factor on stratum corneum water-handling properties. *Journal of Cosmetic Science* 54(3), 289-300.

Wiechers J.W., B. T. (1999): Skin moisturisation and elasticity originate from at least two different mechanisms. *International Journal of Cosmetic Science* 21, 425-435.

Wiechers JW, B. J., De Graaff A. (2003): Identification of another barrier in human stratum corneum: The water barrier. *Journal of Cosmetic Science* 54, 110-111.