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Design of a Lipid-Coated Polymeric Material Mimic Human Skin Surface Properties: a Performing Tool to Evaluate Skin Interaction with Topical Products

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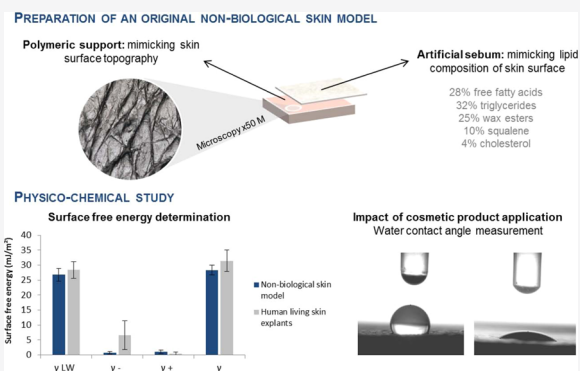
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ABSTRACT: An original nonbiological skin model (NBSM) has been developed to mimic skin surface properties. This skin model presents several improvements when compared to existing ones: it has a topography identical to that of *in vivo* skin and a lipid composition close to that of the skin face. This imparts to the model surface a physicochemistry similar to *in vivo* skin. The concept and design of the skin model are presented, followed by an instrumental characterization performed using 3D microscopy, attenuated total reflectance–Fourier transformed infrared spectroscopy, and contact angle measurements. Surface free energy components are calculated for the NBSM using the Van Oss approach and compared to *ex vivo* experiments performed on human living skin explants. A comparison was made with the commercial skin model VITRO-SKIN, this latter being a reference in terms of the NBSM. The effect of several emollients on skin physicochemistry was investigated *in vivo* and on both nonbiological models. The study focused on several emollients and oils, including silicones and esters, and three emulsions (oil-in-water or water-in-oil). The results obtained confirm the high reliability of the developed model as it shows a skin-like surface behavior. This very interesting tool shows promising results concerning the study of the residual film present after product application or to study skin/product interactions.



INTRODUCTION

Skin is the most extensive and heaviest human organ. It plays a fundamental protective role in our whole body. One of its most important functions is to control the penetration of external compounds and microorganisms into the human body and to limit excessive water loss.¹ This skin barrier property is mainly related to the stratum corneum, its external layer. This layer is composed of dead cells named the corneocytes kept sticking together thanks to intercellular lipids which act as the stratum corneum “cement”.² This external dead layer is in direct contact with the external environment and with any product applied to the skin surface. Both corneocytes and intercellular lipid integrity condition the efficacy of the skin barrier function and the interaction between the skin and its environment.

Studying skin surface properties is consequently of great interest to better understand the roles of corneocytes and lipids in the penetration or permeation phenomenon. These phenomena are relative to passive diffusion and to contact area between skin and external compounds, which are both related to skin physicochemistry.³ This important property describes how skin interacts with its environment and how a compound or product topically applied interacts with skin.

Using the Van Oss approach for surface free energy determination, previous *in vivo* studies showed that skin physicochemistry is greatly impacted by the presence of sebaceous lipids on its surface:^{3,4} the forearm skin, poor in sebum, behaves as a weakly monopolar basic (hydrophobic) surface, whereas the forehead skin, rich in sebum, behaves as a strongly monopolar basic (hydrophilic) surface. These observations are explained by both the lipid amount and composition: sebum is enriched with amphiphilic lipids, especially free fatty acids, which increase the monopolar basic behavior of sebum-rich skins.

When studying the impact of harmful factors on skin physicochemistry, such as UV radiation, urban pollutants, or new cosmetic or pharmaceutical ingredients whose toxicity data are not available, *in vivo* measurements are not feasible because of raw material potential toxicity and regulation. In

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addition, surface free energy determination needs the use of toxic compounds such as diiodomethane which is injurious in the case of skin contact. Consequently, skin models have to be developed and used.

Animal models are available, such as pig skin and rat skin. They present not only similarity with human skin but also differences, above all, concerning lipid compositions⁵ which can impact their physicochemistry. Moreover, in the framework of a cosmetic study, ethical and legal questions are raised: the European Union prohibits all animal-tested products since 2013.⁶

Some alternative skin models have been developed to reduce the use of animals for cosmetic research studies. Reconstructed human skin and human skin explants are probably the most powerful models because their behavior, structure, and composition are close to *in vivo* skin.^{7–9} However, these two models are very expensive and show limited life duration. Consequently, simple, nonbiological surfaces can be used to mimic skin surface properties and to study the frictional and mechanical properties^{10–12} or the adhesion properties.¹³ Nonbiological skin models (NBSMs) such as the VITRO-SKIN (VS) which has been specifically developed to mimic skin surface properties (pH, ionic strength, and physicochemistry) are hence commercially available. For further details, a recent review from authors reports on different NBSM materials and their characteristics.¹⁴ They highlight the interest of measuring and better controlling the surface physicochemistry. The main disadvantage of these commercial products is that their composition remains unknown and unchangeable; thus, they can be considered as “black boxes”. Based on this obvious state-of-the-art, one can consider the need for a new fairly simple, reliable, cheap, and easy to handle synthetic surface exhibiting properties equivalent to *in vivo* human skin.

The aim of the present study is to develop an original NBSM of controlled composition which mimics skin surface properties, chemical composition, and topography, thus imitating its physicochemistry. First, a polymeric material reproducing skin microtopography was prepared. This material was then coated with artificial sebum imitating skin surface lipid composition. Contact angle measurements were performed and compared to *ex vivo* measurements in order to validate the surface physicochemistry of our NBSM. Results were compared to commercial VS as a reference.

In the second step, to deepen the characterization of the new model, physicochemical properties were measured using different emollients and emulsions in order to highlight the interest of the NBSM to study topical application. All the results obtained were compared to *in vivo* skin measurements.

EXPERIMENTAL SECTION

Chemicals Used. Artificial Sebum. Squalene (>98% purity), triolein (glyceryl trioleate 65% purity), and tristearin (glyceryl tristearate, >99% purity) were supplied by Sigma-Aldrich (Saint-Louis, Missouri); cholesteryl oleate (97% purity), cholesterol (95% purity), DL- α -tocopherol (>97% purity), oleic acid (99% purity), chloroform (technical grade), and methanol (technical grade) were supplied by Fisher Scientific SAS (Illkirch, France). Oleyl oleate (Schercemol OLO ester) was supplied by Lubrizol (Oudalle, France). Palmityl palmitate and palmitic and stearic acids were kindly given by Stearinerie Dubois (France).

Liquids for Contact Angle Measurements. Three reference liquids were used to perform surface free energy calculations: ultrapure water (resistivity of 18 M Ω .cm⁻¹ at 25 °C), diiodomethane (analytical grade, 99% purity), and formamide (analytical grade, 99% purity)

(Sigma-Aldrich, Saint-Louis, Missouri). Their surface free energy component values are reported in Table 1.

Table 1. Surface Free Energy Components of Water, Diiodomethane, and Formamide¹⁵

liquid	γ_l (mJ/m ²)	γ_l^{LW} (mJ/m ²)	γ_l^+ (mJ/m ²)	γ_l^- (mJ/m ²)
water	72.8	21.8	25.5	25.5
diiodomethane	50.8	50.8	0.72	0
formamide	58	39	1.92	47.0

Topical Ingredients and Emulsions Used for Physicochemical Study. Four cosmetic grade ingredients were used for the contact angle measurements on the different skin models: cyclopentasiloxane (CPS), a silicone, supplied by Evonik Goldschmidt (Essen, Germany); propanediol dicaprylate (PDC), an ester, kindly given by Stearinerie Dubois (Boulogne-Billancourt, France); and isohehexadecane (IHD), an alkane, supplied by IMCD Group (Cologne, Germany). The chemical structures and physicochemical properties of these ingredients were reported in a previous study.¹⁶ Argan oil (AO), a natural vegetable oil, was supplied by OLVEA Group (Saint-Léonard, France). It is composed¹⁷ of 95% triacylglycerols, ~2% mono/diacylglycerols, and ~2% free fatty acids, and its surface tension at 20 °C was 31.7 mJ/m² (experimental data).

Three complex products for topical application were used: Nivea Crème (NC) (Nivea, Beiersdorf, France), a “moisturizing and nourishing” water-in-oil emulsion, hydroalcoholic gel Assanis Family (GHA) (Assanis, France), a disinfectant aqueous gel, and standard emulsion (SE), a laboratory-made oil-in-water emulsion whose composition and physicochemical properties are described in a previous work.¹⁸

Study Models. Human Living Skin Explants. Human living skin explants were furnished by BIO-EC (Longjumeau, France); they were obtained from plastic surgery of the abdominal area of a 29 year old Caucasian woman, with her consent. To prepare skin explants, the subcutaneous fat was first removed using a scalpel and then, skin (dermis and epidermis) was cut into approximately 2 cm-diameter circles. The explants were placed in survival conditions and maintained in an incubator at 37 °C in 5% CO₂. Half of the BIO-EC’s explant medium was replaced by a fresh one every 2 days. Eight explants were used for the determination of the surface free energy. They were kept in the incubator until contact angle measurements. There were gently wiped before measurements to eliminate the residual explant medium present on the surface.

In Vivo Measurements. *In vivo* measurements were performed on the forearm of a 21 year old Caucasian woman with her consent. The analyzed skin was kept unwashed for 2 h, and no treatment was performed before measurements.

Commercial NBSM. The commercial VS (IMS, Inc., Milford, CT) has been characterized and used in the study. It is a reference in terms of NBSMs for physicochemical studies.

The VS is a synthetic skin model made with proteins and lipids,¹⁹ which mimics skin surface properties such as ionic strength, pH, topography, and critical surface tension. This NBSM is used as a skin substitute for *in vitro* sun protection factor measurements or for the study of emollient spreading.^{19,20} Prior to use, it required to be hydrated according to the standardized protocol developed by IMS. The pieces of VS were placed during 16–24 h at room temperature in a standard closed hydration chamber, which contains 350 g of a mixture water/glycerin 85:15 poured in the bottom.

Preparation of the NBSM Specifically Developed. A new NBSM has been developed for the study. It consists of two distinct parts (see Figure 1).

The first part is a polymeric material which reproduces the skin surface topography. This artificial support is coated with artificial sebum which mimics the lipid composition of the skin face. This NBSM was prepared as described in the following paragraphs.

Preparation of a Polymeric Support Mimicking Skin Surface Topography. A skin print was prepared *in vivo* using the

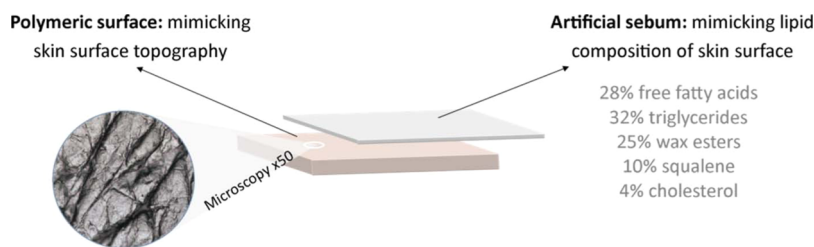


Figure 1. Presentation of the NBSM concept: an artificial skin made with silicone coated with artificial sebum.

biocompatible silicon Body Double (Création-Silicone, Jouy-en-Josas, France) to obtain a silicon surface with a negative relief of the skin. For the present study, the skin print was performed on the forearm of a 26 year old volunteer, with her consent. This skin print was molded back using the silicone Dragon Skin (Dragon Skin 20, Création-Silicone, Jouy-en-Josas, France). This silicon material has already been characterized by Bhushan and Tang²¹ using atomic force microscopy. They highlighted in their work that the Dragon Skin was a good simulation of skin surface properties regarding its adhesive force and frictional behavior after application of topical products.

After mixing together an equal amount of the two components of the kit, the Dragon Skin was degassed under high vacuum for 10 min. The viscous mixture was then poured onto the Body Double skin print and let harden for at least 4 h. The good compatibility between these two products allows a perfect reproduction of the negative skin print.

The polymeric support obtained following this protocol is in the size of the forearm used to prepare the skin print and can be cut into the desired shape. In the present study, the polymeric support was cut into rectangles of dimensions 2 × 5 cm to present a sufficient area to perform all characterizations.

Preparation of the Artificial Sebum. The artificial sebum was prepared following the composition²² described in Table 2. All

The second protocol (referred to as “Protocol 2”) used pure sebum, without any solvent. The solvent was evaporated from the artificial sebum solution. The resulting lipid mixture was placed in an oven at 70 °C until complete liquefaction. The polymeric material was also placed in the oven at 70 °C for 15 min to avoid the temperature difference during deposit. A thin pencil was used to apply a liquid lipid film onto the silicone support. The coated support was then placed back in the oven for 5 min for homogenization. Excess lipids were wiped off using absorbent paper, and the coated silicone was placed again in the oven for 5 min. This last step was crucial as it allowed obtaining a thin homogeneous sebum deposit. The artificial skin model prepared as described was allowed to cool down to room temperature in few minutes.

Stability. In order to realize the different characterizations described below and the physicochemical study, the nonbiological surface model can be stored several weeks under obscurity without modifications.

Surface Characterization of NBSMs. Infrared Spectroscopy.

The Fourier transform infrared (FTIR) spectroscopy spectrum (PerkinElmer, Inc., Waltham, Massachusetts, USA), connected to Spectrum software, was used. Four spectra were recorded for each measurement, using the ATR mode (ZnSe crystal). The range of vibrations was from 4,000 to 650 cm⁻¹.

Optical Microscopy. Surfaces observation was performed using an optical microscope (DMLP/DC 300, Leica Microsystems, Wetzlar, Germany) equipped with a camera and controlled using Leica Application Suite software. All pictures were obtained using the transmission mode, at a magnification of ×50.

Digital Microscopy. A Keyence microscope VHX-1000 (Keyence Corporation TSE, Osaka, Japan) using the VH-Z100R lens at a magnification of ×300 was used. 3D images were recorded in the transmission mode and assembled to obtain a 1600 × 1200 pixel size.

Roughness profiles and parameters were calculated from 3D images of surfaces according to the ISO 25178 norm, using Mountains Map software (Digital Surf SARL, Besançon, France). Three different roughness parameters were reported and studied: S_a , the arithmetic mean height expressed in μm , S_m , the maximum height expressed in μm , and S_{sk} , the dimensionless skewness factor.

Contact Angle Measurement and Surface Free Energy Determination. Advancing Contact Angle Measurements.

Contact angle measurements were performed using a PGX+ (ScanGauge, Gravigny, France) portable goniometer connected to PGPlus software. This goniometer is equipped with a high resolution camera to acquire images, with a specific lightening system associated with a mirror to visualize the droplet. The syringe used for droplet deposition has an intern diameter of 0.77 mm. Measurements were performed at room temperature (20 ± 1.5 °C). Advancing contact angle θ_A is the highest value of the contact angle measured prior to the drop baseline displacement when the drop volume is increased. A small drop was deposited on the surface, and its volume was increased by the addition of five successive drops.^{24,25} The final drop reached a volume of approximately 7 μL . Five pictures were taken immediately after the addition of each drop. The total time of the experiment is around 20 s, so evaporation or penetration of the liquid is neglected. Contact angles from both sides of the drop were calculated using the software program. θ_A was then the maximum value obtained from the mean of both sides of the drop among these five pictures. Advancing

Table 2. Composition Used for the Artificial Sebum

chemical	weight (% w/w)
squalene	10.29
palmityl palmitate	19.42
oleyl oleate	4.85
tristearin	21.36
triolein	10.68
stearic acid	6.87
palmitic acid	6.87
oleic acid	13.74
cholesteryl oleate	1.94
cholesterol	3.88
(\pm)- α -tocopherol	0.10
total	100.00

weighted lipids were dissolved in $\text{CHCl}_3/\text{MeOH}$ 2:1 to reach a concentration of 10 g/L of sebum. The solution was kept in the fridge ($T = 2\text{--}4$ °C) until use. When solvent evaporation was necessary, a rotatory evaporator has been used (Rotavapor R-300, BÜCHI Labortechnik AG, Flawil, Switzerland).

The artificial sebum was then coated onto the previously described polymeric material. Two coating protocols have been tested. They are presented below and discussed in the Results and Discussion section.

Coating Protocols. The first protocol (referred to as “Protocol 1”) used a sebum solution in $\text{CHCl}_3/\text{MeOH}$ (3:7) at a concentration of 20 g/L. Sebum solution was pulverized through a spraying pump on the support.^{11,23} Ten pulverizations at a distance of 5 cm were performed on a 1 cm × 7 cm surface at ambient temperature. Surfaces were then placed in a closed plastic tube to allow slow solvent evaporation and good homogeneity of the lipid coating.

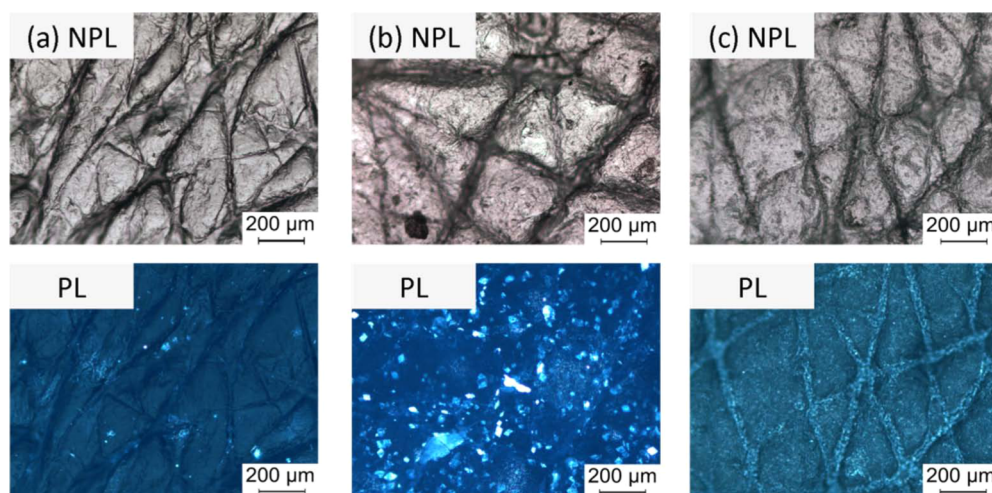


Figure 2. Microscopic images of (a) silicone support and sebum thin layer deposited using (b) Protocol 1 and (c) Protocol 2 (magnification $\times 50$, transmission, nonpolarized light NPL, and polarized light PL).

contact angle measurements for each liquid were performed at least in triplicate for each study model.

Surface Free Energy Determination. To determine the surface free energy of solid surfaces, one of the most recent approaches has been developed by Van Oss and co-workers.^{3,26,27} They suggest that solid and liquid surface free energies can be divided into two components: the Lifschitz–van der Waals component (γ^{LW}), which corresponds to the van der Waals interactions (Keesom, Debye, London), and the acid–base component (γ^{AB}), which corresponds to the acidic and basic interactions, according to the Lewis concept (1)

$$\gamma = \gamma^{LW} + \gamma^{AB} \quad (1)$$

This acid–base component can be expressed by the geometrical mean of two parameters: γ^+ (electron acceptor) and γ^- (electron donor) (2)

$$\gamma^{AB} = 2\sqrt{\gamma^+\gamma^-} \quad (2)$$

This last component includes the hydrogen bonding and the π –electron interactions.

Surface free energy can be calculated from contact angle measurements using the Young Equation (3)

$$\gamma_L \cos \theta = \gamma_S - \pi_e - \gamma_{SL} \quad (3)$$

Where γ_L is the liquid surface tension, θ is the contact angle, γ_S is the solid surface free energy, π_e is the film pressure of the liquid, and γ_{SL} is the interfacial surface free energy. “L” and “S” refer to liquid and solid, respectively. π_e can be neglected for low energy solids such as skin.³

According to Van Oss, the interfacial surface free energy can be expressed as follow (4)

$$\gamma_{SL} = \gamma_S^{LW} + \gamma_L^{LW} - 2\sqrt{\gamma_S^{LW}\gamma_L^{LW}} + 2(\sqrt{\gamma_S^+\gamma_S^-} + \sqrt{\gamma_L^+\gamma_L^-} - \sqrt{\gamma_S^+\gamma_L^-} - \sqrt{\gamma_S^-\gamma_L^+}) \quad (4)$$

Thus, the Young equation can be written (5)

$$(1 + \cos \theta)\gamma_L = 2\sqrt{\gamma_L^{LW}\gamma_S^{LW}} + 2\sqrt{\gamma_L^+\gamma_S^-} + 2\sqrt{\gamma_L^-\gamma_S^+} \quad (5)$$

Following (5), the surface free energy of the solid surface can be determined using at least three reference liquids of known component values.

The Van Oss model has been defined for a smooth and homogeneous surface. For a rough surface, the roughness coefficient (6) defined by Wenzel²⁸ should be used to adjust the contact angle value (7)

$$r = \frac{\text{real surface area}}{\text{appearing surface area}} \geq 1 \quad (6)$$

$$\cos \theta^* = r \cos \theta \quad (7)$$

where r is the roughness coefficient, θ^* is the measured contact angle, and θ is the real contact angle.

Nevertheless, some studies dealing with the effect of roughness on the wettability of the random surface such as skin show that this relation does not correctly describe the real contact angle.^{3,29,30} Moreover, we try to reduce the impact of roughness on our results using surfaces of equivalent topography. That is why skin roughness has not been taken into account for this study.

Physicochemical Study. Ingredient Contact Angle on Untreated Surfaces. The advancing contact angles of the four ingredients—the silicone, the ester, the alkane, and the AO—were measured in triplicate on each study models. Drop deposition protocol was the same as the one described previously.

Water Contact Angle after Product Application. The four cosmetic ingredients (50 μL each) and the three topical complex products were manually spread on 10 cm^2 surface areas by 40 rotations. Water contact angle measurements were performed 1 and 3 min after application, in triplicate, to characterize the properties of the residual film.

RESULTS AND DISCUSSION

Optimization of Sebum Coating on the NBSM. Two protocols were tested to coat the sebum thin layer on the silicone support. Sebum thin layers were visualized using an optical microscope in the transmission mode (Figure 2).

The presence of lipids on the polymeric support gives a granular aspect to the relief (Figure 2b,c). Using Protocol 1, lipids clearly crystallize, as evidenced in Figure 2b: diamond-shaped crystals are visible under polarized light. This crystallization, probably because of solvent evaporation, gives a glitter aspect to the surface, which is not appropriate for the lipid coating aspect. This crystallization is not observed for Protocol 2 which exhibits an apparent homogeneous sebum layer over the entire surface (Figure 2c).

In order to check the deposition protocol reliability, surface free energy was determined before and after sebum coating onto the silicone surface. Results are summarized in Figure 3. The graph includes additional data resulting from human living skin explant measurements.

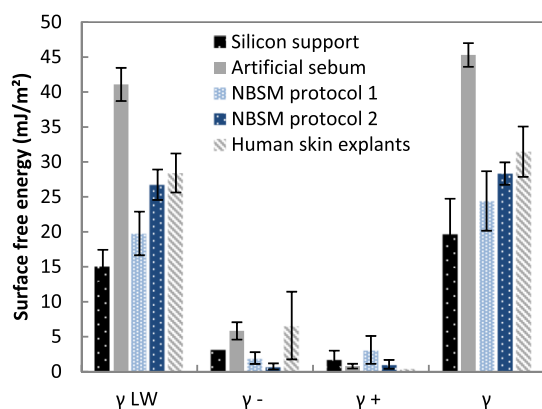


Figure 3. Surface free energy parameters as determined for the different surfaces: the silicone surface, the artificial sebum, the NBSM prepared using Protocol 1, the NBSM prepared using Protocol 2, and human living skin explants.

Unsurprisingly, knowing the low surface tension of silicone polymers, the silicone surface obtained shows a low surface free energy (γ) when compared to *ex vivo* skin: 19.7 ± 5.0 mJ/m² for silicone against 31.5 ± 3.6 mJ/m² for *ex vivo* skin. Artificial sebum properties demonstrate that this coating is able to increase the LW (Lifschitz–van der Waals) component value once applied on a given surface. Its high LW component value ($\gamma_{LW\text{sebum}} = 41.1 \pm 2.4$ mJ/m²) can be explained by the composition of this mixture of lipids. Indeed, triglycerides and other constitutive lipids with a long carbon chain will highly interact with each other, increasing surface cohesiveness. This cohesion will increase surface energy through apolar interactions. Thus, the addition of the sebum thin layer on the silicone allows reaching a value of 26.7 ± 2.2 mJ/m² for the LW component on the NBSM prepared with Protocol 2, in good agreement with the value of 28.4 ± 2.8 mJ/m² calculated on human living skin explants. Noteworthy, these results are in accordance with literature data calculated from *in vivo* measurements.³

The values obtained for the NBSM following Protocol 2 for sebum deposition are therefore consistent with the measurements carried out with the human living skin explants. It is interesting to note that the basic component (γ^-) is slightly lower for the coated NBSM when compared to human living skin explants. Nevertheless, this component depends on the type of skin: according to Mavon *et al.*,^{3,4} sebum-enriched skins show higher surface free energy values than the poor sebum area. This difference for the surface free energy is due to the higher basic component value for sebum-rich skins. They assumed that this high basic component can be related to the sebaceous lipid level and to its amphiphilic lipid content, specifically free fatty acids. However, the artificial sebum studied here did not show a particularly high basic component: $\gamma_{\text{sebum}}^- = 5.8 \pm 1.2$ mJ/m². Consequently, the NBSM did not reach as high γ^- as found in rich sebum *in vivo* skins. Both protocols show low values for the basic component: 1.9 ± 0.8 mJ/m² for Protocol 1 and 0.7 ± 0.4 mJ/m² for Protocol 2. The NBSM physicochemistry is therefore much closer to the poor sebum skin surface area as forearm skin rather than forehead skin.

The results clearly indicate that Protocol 1 is less efficient than Protocol 2 to reach properties close to the *ex vivo* human skin. Indeed, one can observe that surfaces prepared using Protocol 1 show higher values for the acidic component ($3.1 \pm$

2.0 mJ/m²), in comparison with the *ex vivo* skin (0.5 ± 0.5 mJ/m²). Then, the LW component determined for Protocol 1 (19.8 ± 3.1) is lower than the one obtained *ex vivo*. Protocol 2 is so much appropriate to mimic skin surface physicochemistry. In addition, the absence of the solvent in Protocol 2 is undoubtedly advantageous for environmental concerns and it also prevents the risk of dissolution of the polymeric material once covered by the sebum. Moreover, light microscopy proves that sebum distribution was more homogeneous and that lipids crystallization was limited with Protocol 2. For all these reasons, the second protocol was selected and applied for the rest of the study.

The lipids coated over the support surface were then characterized by infrared spectroscopy (Figure 4).

Vibrations associated with the artificial sebum are visible in the NBSM infrared spectrum. They are indicated by the black arrows in Figure 4a, and its associated wavenumbers are reported in Table 3 and compared to *in vivo* data.

Wavenumbers associated with CH₂ symmetric stretching (Table 3) give information about lipid chain conformational packing. According to Mendelsohn *et al.*,³² lipid chain packing evolves into the stratum corneum depth. Even if the solid lipid structures in the stratum corneum are not only built up by the sebum lipids, as in our NBSM, it is possible to rely on these results. Through the extreme surface (from 0 to 4 μ m), the CH₂ symmetric stretching wavenumber evolves from 2853 to 2849 cm⁻¹, which corresponds to disordered and hexagonal chain packing. This is due to the specific lipid composition enriched in unsaturated lipids that cover the skin. Deeper into the stratum corneum, saturated lipids are predominant and show a highly ordered orthorhombic packing with associated wavenumbers between 2849 and 2847 cm⁻¹. This highly ordered packing provides the stratum corneum its water barrier function. *In vivo* FTIR spectral data recorded on the forehead show a disordered chain packing, corresponding to a liquid state.³² Artificial sebum and NBSM FTIR spectra show a hexagonal type chain packing, explained by the mixture of saturated and unsaturated lipids chains used. This is consequently an intermediary packing between the liquid lipid state and highly ordered lipid organization (wavenumber < 2849 cm⁻¹). This conformation is hence consistent with the extreme surface lipid chain packing between 2853 and 2849 cm⁻¹ described by Mendelsohn.

Surface Property Characterization of VS and the NBSM. The NBSM developed here was then compared with VS that is frequently used for studying skin surface properties.

Infrared Spectroscopy. Infrared spectroscopy was first carried out to compare the two NBSMs. Spectra obtained on both models are stacked with *in vivo* spectra recorded on the forehead and on the forearm (Figure 4b).

As expected, Figure 4b shows distinct spectra which reflect differences in composition of the surfaces. The NBSM is enriched in sebaceous lipids, whose associated absorption vibrations (2914 and 2849 cm⁻¹) lie between the ones of the *in vivo* forearm and forehead, whereas they are absent on the VS. VS exhibits vibrations associated to ceramides³¹ (1631 and 1553 cm⁻¹). These lipids are involved in the stratum corneum lipid organization and consequently in the skin barrier function.^{33,34} For the NBSM, where ceramides are absent, we have shown previously that we are able to reach a surface physicochemistry very close to the *ex vivo* skin surface using only sebaceous lipids. The impact of ceramides on skin surface physicochemistry is negligible when compared to sebaceous

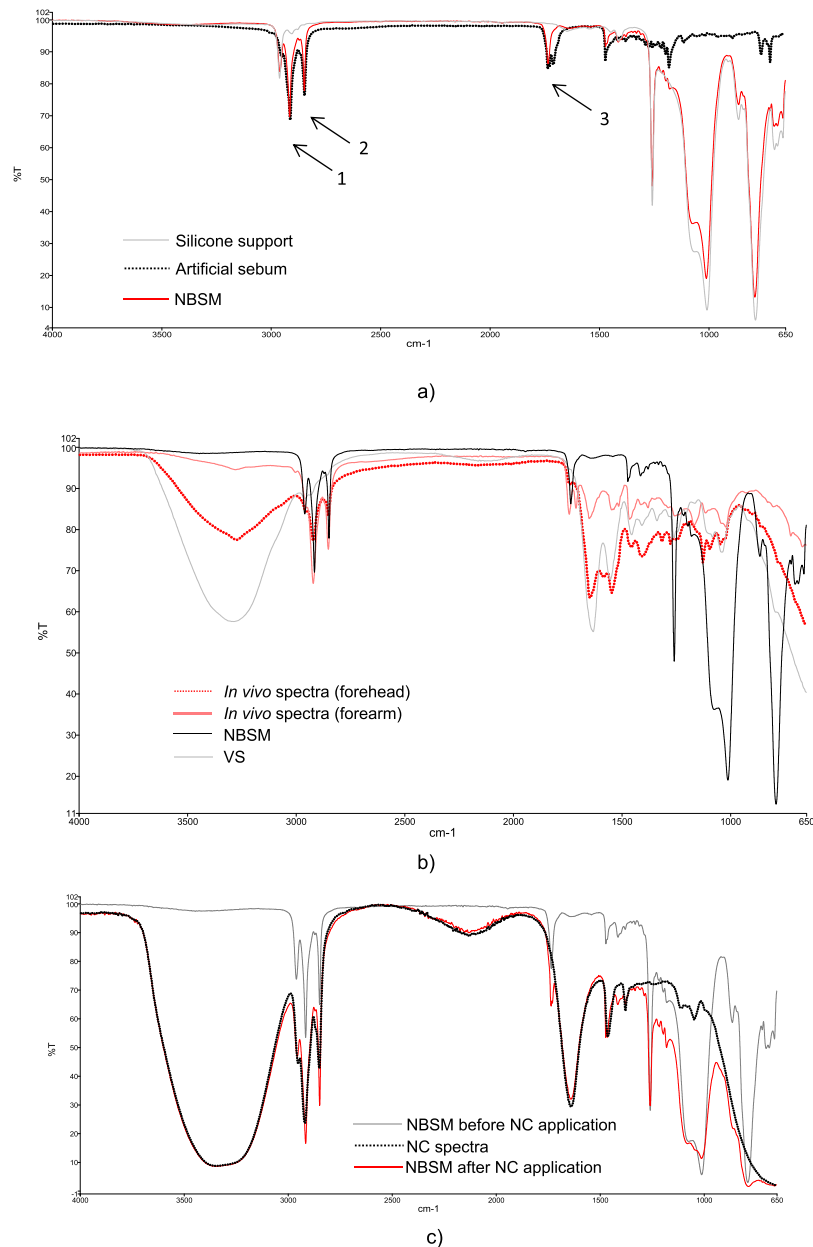


Figure 4. Infrared spectra recorded (a) before/after sebum coating using Protocol 2; (b) *in vivo*, on the VS and on the NBSM; and (c) on NBSM before and 3 min after application of NC (W/O emulsion).

Table 3. Wavenumbers (cm⁻¹) for *In Vivo* Skin, the Artificial Sebum, and the NBSM

peak number	<i>in vivo</i> skin	artificial sebum	NBSM	chemical structure associated ^{31,32}
1	2922.54	2914.65	2916.71	CH ₂ asymmetric stretch
2	2853.14	2849.06	2849.74	CH ₂ symmetric stretch
3	1741.63 and 1710.90	1735.33 and 1710.27	1735.13	C=O esters (1720–1790) and fatty acids (1690–1720)

lipids. That is why the addition of ceramides in the case of the present physicochemical study was not relevant.

VS is also well hydrated as highlighted by the important vibration associated to –OH structures ($\nu = 3286 \text{ cm}^{-1}$). This hydration state is more important than the one of both *in vivo* systems when comparing FTIR spectra. This can be a source of

divergence concerning physicochemical behavior of VS and *in vivo* skin.

3D Microscopy. 3D microscopy gives access to surface topography of both NBSMs and helps to study their roughness. 3D pictures obtained are presented in Figure Sa,b, and roughness profiles are summarized in Figure Sc,d.

Roughness parameters are summarized in Table 4. Results highlight that both models show S_a and S_z in the order of magnitude of *in vivo* skin. An important difference between the NBSM and the VS concerns the skewness factor S_{sk} : $S_{sk_NBSM} = -0.739$ against 0.648 for S_{sk_VS} . This important parameter describes surface morphology: a positive S_{sk} value corresponds to a surface showing peaks and protuberance projecting above the average height, whereas a negative S_{sk} value corresponds to a tray surface with deep scratches and pores. These values are in accordance with visual observations shown in Figure Sa,b.

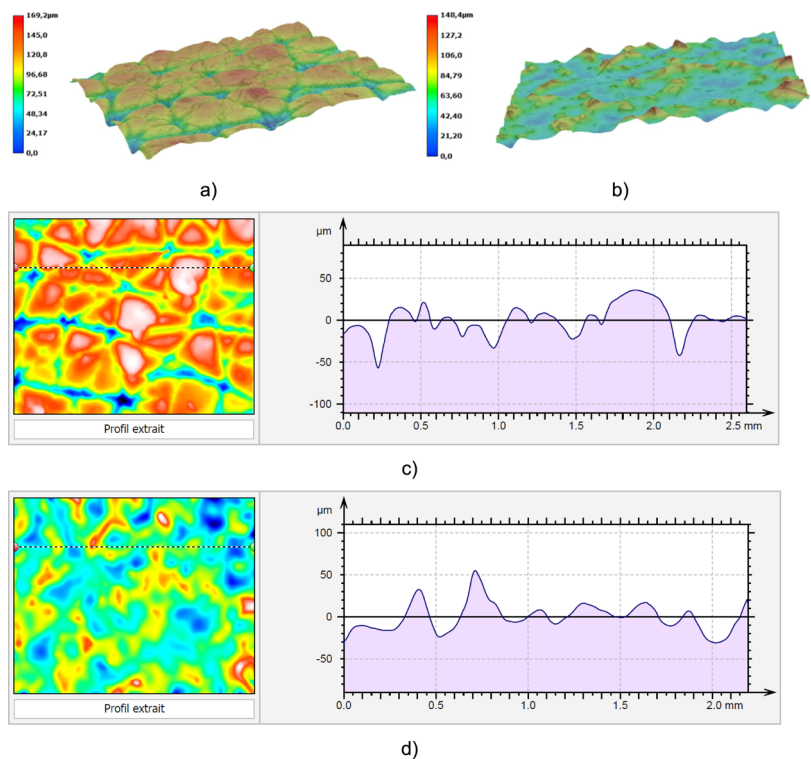


Figure 5. (a) 3D microscopy images (magnification $\times 300$) of NBSM; (b) 3D microscopy images (magnification $\times 300$) of VS; (c) roughness profiles of NBSM; and (d) roughness profiles of VS.

Table 4. Roughness Parameters Calculated on the NBSM and on VS Where S_a : Arithmetic Mean Height and S_z : Maximum Height; S_{sk} : Skewness Factor

roughness parameters	<i>in vivo</i> ^{11,35} (volar forearm)	NBSM	VS
S_a (μm)	[17; 20]	23.4	15.4
S_z (μm)	[119; 125]	169	148
S_{sk}	[−0.46; −0.91]	−0.739	0.648

Consequently, parameter S_{sk} is clearly discriminant between both nonbiological models.

In addition, *in vivo* literature data report negative S_{sk} values between −0.46 and −0.91. Therefore, the skewness factor of the NBSM is in good agreement with the *in vivo* parameter.

The NBSM mimics forearm skin relief with much more accuracy than VS. As a consequence, in the aim to compare contact angle measurements performed on the *in vivo* volar forearm, the NBSM appears more suitable because surface roughness has a significant impact on contact angle measurements.²⁸

To summarize, the NBSM developed in the present work shows chemical composition and topographic properties close to the *ex vivo* skin. Moreover, as shown in the previous paragraph, the sebum coating has been optimized to be as close as possible to skin physicochemistry. All these results demonstrate that the NBSM is a relevant skin model to mimic a large range of skin surface properties.

In order to show the interest of the NBSM, complementary measurements have been performed to evaluate its interactions with emollient and its physicochemical behavior after application of products. For this study, contact angles between water, emollients, and *in vivo* skin were compared with measurements on both NBSMs (VS and NBSM). However, for discussion, we had to compare surface free energy data with *ex*

vivo-calculated values as it was not possible to determine the surface free energy of the *in vivo* skin of our panelist because of the toxicity of the reference liquids used.

Physicochemical Study of Topical Ingredients and Emulsions: Comparison with In Vivo Skin. *Study of Emollient Spreading.* Contact angle measurements are part of instrumental methods which are used to study spreading properties of emollients on skin.^{16,19} Spreading of emollients is an important purpose not only for the sensory quality of a topical product¹⁶ but also for its efficacy, above all, for sunscreens.^{36–38} Indeed, the sun protection factor is on the one hand due to solar filters present in emulsions and on the other hand due to the homogeneity of the residual film once the product is spread onto the skin surface. Thus, among other parameters responsible for the film formation and homogeneity, the spreading appears of primary importance. In order to evaluate spreading properties of topical ingredients, contact angle measurements were performed with four ingredients: AO, IHD, PDC, and CPS on the different surfaces studied herein. Results obtained on the NBSM, on the VS, and *in vivo* are presented in Figure 6a.

From a general point of view, the contact angle measured using the four different ingredients appear very similar between the NBSM and *in vivo* surfaces. On the opposite, advancing contact angles as measured for the different emollients on the VS are lower when compared to both *in vivo* and NBSM surfaces. These results mean that the NBSM surface, specifically developed in the present work, interacts with products in the same way as the *in vivo* skin. The only difference concerns IHD, which shows more affinity with *in vivo* skin than with the NBSM. This can be explained by the slightly higher skin LW component, as calculated *ex vivo* and confirmed in the literature,³ compared to the NBSM, thus inducing higher interactions with the apolar IHD. However,

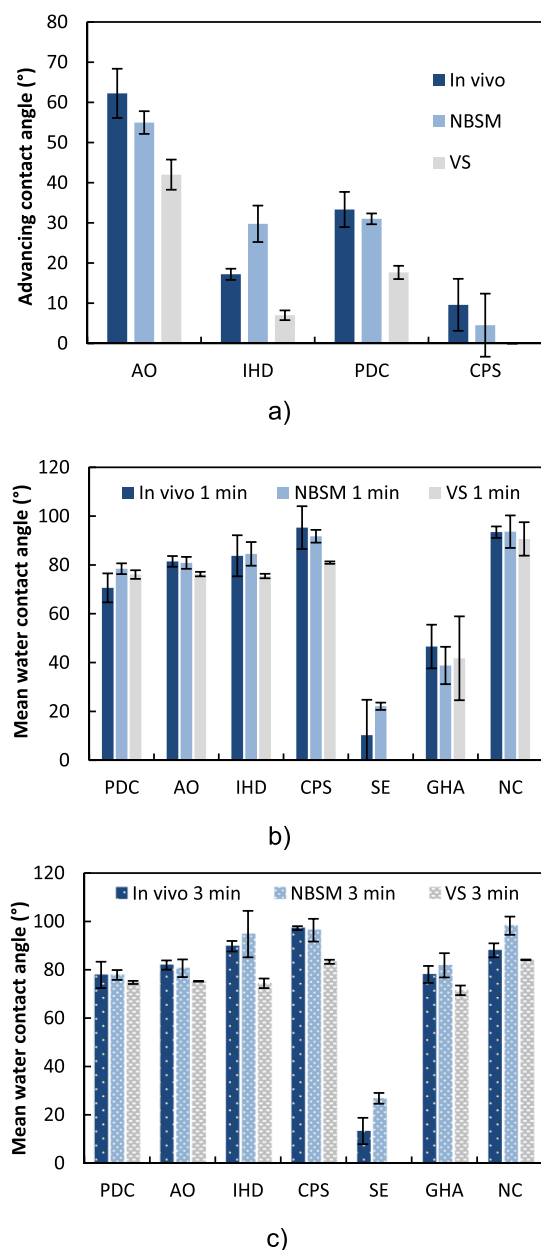


Figure 6. Advancing contact angle values (a) measured for AO, IHD, PDC, and CPS; (b) for water determined 1 min after product application; and (c) 3 min after product application. Three surfaces are compared: *in vivo* skin, the NBSM, and the VS.

this NBSM undoubtedly constitutes an efficient support to evaluate emollient spreading if compared to VS. Indeed, lower contact angles measured on VS indicate that each ingredient shows more affinity with the VS than with *in vivo* skin. As a consequence, spreading of emollients is enhanced because of a higher surface free energy for VS ($\gamma_{VS} = 35.8 \text{ mJ/m}^2$) when compared to *ex vivo* skin and the NBSM. The hydration state of the VS may be responsible for this result as the presence of water classically tends to increase the surface free energy value. According to these results, the NBSM interestingly appears as a relevant tool to study the residual film present on the skin surface after topical application. The physicochemical characterization of residual films is presented below.

Characterization of the Residual Film after Product Application. Characterization of the residual film of an

ingredient or an emulsion on skin is a very interesting topic as its homogeneity, composition, and stability greatly impact skin surface properties and, consequently, the efficacy of actives and products. To study the physicochemistry of residual films, the water contact angle has been measured on VS, on the NBSM, and *in vivo* at two distinct times (1 and 3 min) once a series of products applied following the protocol described in the Experimental Section. Results are presented in Figure 6b,c.

Water contact angles measured 1 min after surface treatment with emollients are classified as follows: $\theta_{\text{water}}/\text{PDC} < \theta_{\text{water}}/\text{AO} < \theta_{\text{water}}/\text{IHD} < \theta_{\text{water}}/\text{CPS}$ for both *in vivo* skin and the NBSM, whereas $\theta_{\text{water}}/\text{IHD} < \theta_{\text{water}}/\text{PDC} \approx \theta_{\text{water}}/\text{AO} < \theta_{\text{water}}/\text{CPS}$ for VS. Before product application, $\theta_{\text{water}} \text{ in vivo} = 93.2 \pm 7.8^\circ$, $\theta_{\text{water}} \text{ NBSM} = 114.2 \pm 5.8^\circ$, and $\theta_{\text{water}} \text{ VS} = 104.2 \pm 11.8^\circ$. Globally, ingredients reduce water contact angle values for each surface.

For NBSM and *in vivo* skin, results obtained are in accordance with surface tension values of each ingredient ($\gamma_{\text{PDC}} = 33.1 \text{ mJ/m}^2$, $\gamma_{\text{AO}} = 31.7 \text{ mJ/m}^2$, $\gamma_{\text{IHD}} = 24.4 \text{ mJ/m}^2$, $\gamma_{\text{CPS}} = 18.0 \text{ mJ/m}^2$), the lower the ingredient surface tension, the higher the water contact angle. Indeed, the emollient residual film modifies the skin composition and surface tension. As an example, CPS with a low surface tension decreases skin surface energy.

Concerning the complex formulations, the differences between the three surfaces, namely, *in vivo*, NBSM, and VS, appear less pronounced. The W/O emulsion (NC) exhibits the highest values of water contact angle, followed by the hydroalcoholic gel, while SE obtained the lowest values. Their composition can explain the values obtained: SE and GHA contain more than 70% of water and isopropyl alcohol, respectively, which makes the skin more hydrophilic after application, whereas for the inverse emulsion NC, its continuous oily phase is more important and makes the skin more hydrophobic.

The skin model VS displays surprisingly a null water contact angle for the SE. Differences observed for VS in the measurements after emulsion application can be due to its absorption capacity and its high hydration, as explained previously. Water from the deposit drop can interact with the high amount of water contained in VS, which decreased contact angle values.

The water contact angle measured 3 min after treatment can be useful to evaluate the evolution of a residual film on skin for a longer time. For instance, the GHA shows an important increase in water contact angle between 1 and 3 min after application. This is probably the result of isopropyl alcohol evaporation. In this case, only nonvolatile and nonpenetrating ingredients of the product remain on the skin 3 min after application. In opposition, the SE residual film did not significantly evolve after 3 min, which means that both penetration and evaporation of compounds are not achieved.

Whatever the type of ingredient (polar or apolar oil, ester, and silicon) or emulsion used, advancing water contact angles determined on the NBSM and *in vivo* remain very close. VS is also performing, but to a lesser extent, because of few differences with *in vivo* measurements. VS also shows less variation among products, above all, 3 min after application. To sum up, results obtained in the present section with the NBSM are very promising in the aim to study residual films of topical products on the surface of the skin and to highlight their effects on surface skin physicochemical properties.

The final example of application concerns the use of the NBSM to characterize the residual film after product application in terms of chemical composition, as shown in infrared spectra in Figure 4c before and after NC application to the skin.

According to the ingredient applied, we can highlight its presence on the NBSM surface by following one or more vibrations associated with their original spectrum. In this example, the vibration at 1639 cm^{-1} is characteristic of the presence of the NC emulsion on the surface. Such an illustration highlights how it can be very interesting to study the chemical composition of the residual film after application and its evolution over time using the NBSM surface. Another perspective covers the investigation of the homogeneity of a residual film by means of IR microscopy or Raman microscopy for instance.³⁹

Surprisingly, the investigations dealing with the impact of topical products on skin surface physicochemistry are not numerous in the literature. However, it is a fundamental point to highlight and understand the interactions between the skin surface and a topical product. The use of such a skin surface model can help to fulfill this lack of data by standardizing the complex skin surface. It is a way to deepen the comprehension of phenomena involved in skin permeation enhancement, topical drug delivery, or skin adhesive properties for instance. In addition, the skin surface model developed in the present work has the great advantage to present a lipid coating, allowing the study of the interactions between human skin lipids and topical products, which represent an interesting perspective.

Moreover, in the aim to study a new ingredient whose toxicity has not been evaluated, the use of the NBSM is of great interest: it permits studying properties of ingredients without any safety trouble and with no particular technical difficulties.

CONCLUSIONS

This study describes the design, optimization, characterization, and use of an original NBSM which mimics skin surface properties, namely, chemical composition, topography, and physicochemical properties. This NBSM consists of an artificial material prepared with silicone and mold from an *in vivo* skin print, whose surface is coated with an artificial lipid mixture mimicking human sebum composition. Coating protocol has been tuned to be as close as possible to *in vivo* and *ex vivo* skin general characteristics. The layer of lipids coated on the silicone support has been characterized using IR spectroscopy, and the resulting skin model surface has been studied using 3D microscopy. When compared to existing models, this NBSM has the advantage to reproduce the real microtopography of the human skin surface. The study focuses on a volar forearm relief but other body part skin prints such as the forehead or back can be used. Present NBSM technology is therefore fully versatile, and it represents high potentiality as a way to deepen the understanding of the skin microrelief impact on its surface physicochemistry, on product interactions with skin surface, and spreading behavior onto the skin.

Additional studies have been performed on this NBSM to present its potential uses to characterize the residual film present on the surface of the skin after topical application. This physicochemical study highlights the similarity between the *in vivo* skin surface and NBSM behavior in contact with emollients and emulsions. This can be of great interest to

develop the knowledge of a residual film after application of raw materials or products such as gels or emulsions. This model could be a tool that, combined with other analyses, would be a key area not only for sensory characterization but also for efficacy assessments. As an outlook, a control of the mechanical properties and the permeability of the silicone support can be important parameters to improve the tactile perceptions of the surface, in order to better mimic the product application onto the skin.

This nonbiological surface is of great interest not only to study efficacy of new products on the surface of the skin but also for more fundamental research studies. Lipid composition can be tuned by adding some epidermal lipids to mimic cutaneous disorders linked to a modification in skin lipids such as atopic dermatitis or symptoms of dry skin.⁴⁰ This can obviously constitute a novel way to contribute to the characterization of these skin disorders, through their skin surface physicochemical consequences.

This NBSM skin model may also be used to study the impacts of many external factors such as UV radiation, ozone, or urban pollutants on skin surface physicochemistry and to understand adhesion of particles or microorganisms.

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Notes

The authors declare no competing financial interest.

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REFERENCES

- (1) Brisson, P. Percutaneous Absorption. *Can. Med. Assoc. J.* **1974**, *110*, 1182–1185.
- (2) Proksch, E.; Brandner, J. M.; Jensen, J.-M. The Skin: An Indispensable Barrier. *Exp. Dermatol.* **2008**, *17*, 1063–1072.

- (3) Mavon, A.; Zahouani, H.; Redoules, D.; Agache, P.; Gall, Y.; Humbert, P. Sebum and Stratum Corneum Lipids Increase Human Skin Surface Free Energy as Determined from Contact Angle Measurements: A Study on Two Anatomical Sites. *Colloids Surf., B* **1997**, *8*, 147–155.
- (4) Mavon, A.; Redoules, D.; Humbert, P.; Agache, P.; Gall, Y. Changes in Sebum Levels and Skin Surface Free Energy Components Following Skin Surface Washing. *Colloids Surf., B* **1998**, *10*, 243–250.
- (5) Stahl, J.; Niedorf, F.; Kietzmann, M. Characterisation of Epidermal Lipid Composition and Skin Morphology of Animal Skin *Ex Vivo*. *Eur. J. Pharm. Biopharm.* **2009**, *72*, 310–316.
- (6) Merenyi, S. COSMETICS: Regulation (EC) No 1223/2009, 2014.
- (7) Bouwstra, J. A.; Gooris, G. S.; Weerheim, A.; Kempenaar, J.; Poncet, M. Characterization of Stratum Corneum Structure in Reconstructed Epidermis by X-Ray Diffraction. *J. Lipid Res.* **1995**, *36*, 496–504.
- (8) Lerebour, G.; Cupferman, S.; Cohen, C.; Bellon-Fontaine, M. N. Comparison of Surface Free Energy between Reconstructed Human Epidermis and in Situ Human Skin. *Skin Res. Technol.* **2000**, *6*, 245–249.
- (9) Tfayli, A.; Bonnier, F.; Farhane, Z.; Libong, D.; Byrne, H. J.; Baillet-Guffroy, A. Comparison of Structure and Organization of Cutaneous Lipids in a Reconstructed Skin Model and Human Skin: Spectroscopic Imaging and Chromatographic Profiling. *Exp. Dermatol.* **2014**, *23*, 441–443.
- (10) Nachman, M.; Franklin, S. E. Artificial Skin Model Simulating Dry and Moist *in Vivo* Human Skin Friction and Deformation Behaviour. *Tribol. Int.* **2016**, *97*, 431–439.
- (11) Gerhardt, L.-C.; Schiller, A.; Müller, B.; Spencer, N. D.; Derler, S. Fabrication, Characterisation and Tribological Investigation of Artificial Skin Surface Lipid Films. *Tribol. Lett.* **2009**, *34*, 81–93.
- (12) Dąbrowska, A. K.; Rotaru, G.-M.; Derler, S.; Spano, F.; Camenzind, M.; Annaheim, S.; Stämpfli, R.; Schmid, M.; Rossi, R. M. Materials Used to Simulate Physical Properties of Human Skin. *Skin Res. Technol.* **2016**, *22*, 3–14.
- (13) Charkoudian, J. C. A Model Skin Surface for Testing Adhesion to Skin. *J. Soc. Cosmet. Chem.* **1988**, *39*, 225–234.
- (14) Eudier, F.; Savary, G.; Grisel, M.; Picard, C. Skin surface physico-chemistry: Characteristics, methods of measurement, influencing factors and future developments. *Adv. Colloid Interface Sci.* **2019**, *264*, 11–27.
- (15) Janczuk, B.; Chibowski, E.; Bruque, J. M.; Kerkeb, M. L.; Caballero, F. G. On the Consistency of Surface Free Energy Components as Calculated from Contact Angles of Different Liquids: An Application to the Cholesterol Surface. *J. Colloid Interface Sci.* **1993**, *159*, 421–428.
- (16) Savary, G.; Grisel, M.; Picard, C. Impact of Emollients on the Spreading Properties of Cosmetic Products: A Combined Sensory and Instrumental Characterization. *Colloids Surf., B* **2013**, *102*, 371–378.
- (17) Charrouf, Z.; Guillaume, D. Argan Oil: Occurrence, Composition and Impact on Human Health. *Eur. J. Lipid Sci. Technol.* **2008**, *110*, 632–636.
- (18) Gilbert, L.; Picard, C.; Savary, G.; Grisel, M. Impact of Polymers on Texture Properties of Cosmetic Emulsions: A Methodological Approach. *J. Sens. Stud.* **2012**, *27*, 392–402.
- (19) Douguet, M.; Picard, C.; Savary, G.; Merlaud, F.; Loubat-bouleuc, N.; Grisel, M. Spreading Properties of Cosmetic Emollients: Use of Synthetic Skin Surface to Elucidate Structural Effect. *Colloids Surf., B* **2017**, *154*, 307–314.
- (20) Jermann, R.; Toumiat, M.; Imfeld, D. Development of an *in vitro* efficacy test for self-tanning formulations. *Int. J. Cosmet. Sci.* **2002**, *24*, 35–42.
- (21) Bhushan, B.; Tang, W. Surface, Tribological, and Mechanical Characterization of Synthetic Skins for Tribological Applications in Cosmetic Science. *J. Appl. Polym. Sci.* **2011**, *120*, 2881–2890.
- (22) Stefaniak, A. B.; Harvey, C. J.; Wertz, P. W. Formulation and Stability of a Novel Artificial Sebum under Conditions of Storage and Use. *Int. J. Cosmet. Sci.* **2010**, *32*, 347–355.
- (23) Jones, S.; Rio, E.; Cazeneuve, C.; Nicolas-Morgantini, L.; Restagno, F.; Luengo, G. S. Tribological Influence of a Liquid Meniscus in Human Sebum Cleaning. *Colloids Surf., A* **2016**, *498*, 268–275.
- (24) Timmons, C. O.; Zisman, W. A. The Effect of Liquid Structure on Contact Angle Hysteresis. *J. Colloid Interface Sci.* **1966**, *22*, 165–171.
- (25) Drelich, J. Guidelines to Measurements of Reproducible Contact Angles Using a Sessile-Drop Technique. *Surf. Innovations* **2013**, *1*, 248–254.
- (26) Van Oss, C. J.; Good, R. J.; Chaudhury, M. K. Additive and Nonadditive Surface Tension Components and the Interpretation of Contact Angles. *Langmuir* **1988**, *4*, 884–891.
- (27) Van Oss, C. J.; Chaudhury, M. K.; Good, R. J. Interfacial Lifshitz-van Der Waals and Polar Interactions in Macroscopic Systems. *Chem. Rev.* **1988**, *88*, 927–941.
- (28) Wenzel, R. N. Resistance of Solid Surfaces to Wetting by Water. *Ind. Eng. Chem.* **1936**, *28*, 988–994.
- (29) Oliver, J. P.; Huh, C.; Mason, S. G. An Experimental Study of Some Effects of Solid Surface Roughness on Wetting. *Colloids Surf.* **1980**, *1*, 79–104.
- (30) Cazabat, A. M.; Stuart, M. A. C. Dynamics of Wetting: Effects of Surface Roughness. *J. Phys. Chem.* **1986**, *90*, 5845–5849.
- (31) Prasch; Knübel; Schmidt-Fonk; Ortanderl; Nieveler; Förster. Infrared Spectroscopy of the Skin: Influencing the Stratum Corneum with Cosmetic Products. *Int. J. Cosmet. Sci.* **2000**, *22*, 371–383.
- (32) Mendelsohn, R.; Flach, C. R.; Moore, D. J. Determination of Molecular Conformation and Permeation in Skin via IR Spectroscopy, Microscopy, and Imaging. *Biochim. Biophys. Acta, Biomembr.* **2006**, *1758*, 923–933.
- (33) Bouwstra, J. A.; Cheng, K.; Gooris, G. S.; Weerheim, A.; Poncet, M. The Role of Ceramides 1 and 2 in the Stratum Corneum Lipid Organisation. *Biochim. Biophys. Acta, Lipids Lipid Metab.* **1996**, *1300*, 177–186.
- (34) Bouwstra, J. A.; Dubbelaar, F. E. R.; Gooris, G. S.; Weerheim, A. M.; Poncet, M. The Role of Ceramide Composition in the Lipid Organisation of the Skin Barrier. *Biochim. Biophys. Acta, Biomembr.* **1999**, *1419*, 127–136.
- (35) Lagarde, J. m.; Rouvrais, C.; Black, D. Topography and Anisotropy of the Skin Surface with Ageing. *Skin Res. Technol.* **2005**, *11*, 110–119.
- (36) Draelos, Z. D. Therapeutic Moisturizers. *Dermatol. Clin.* **2000**, *18*, 597–607.
- (37) Fageon, L.; Moyal, D.; Coutet, J.; Candau, D. Importance of Sunscreen Products Spreading Protocol and Substrate Roughness for *in Vitro* Sun Protection Factor Assessment. *Int. J. Cosmet. Sci.* **2009**, *31*, 405–418.
- (38) Vergou, T.; Patzelt, A.; Schanzer, S.; Meinke, M. C.; Weigmann, H.-J.; Thiede, G.; Sterry, W.; Lademann, J.; Darvin, M. E. Methods for the Evaluation of the Protective Efficacy of Sunscreen Products. *Skin Pharmacol. Physiol.* **2013**, *26*, 30–35.
- (39) Sohn, M.; Buehler, T.; Imanidis, G. Repartition of Oil Miscible and Water Soluble UV Filters in an Applied Sunscreen Film Determined by Confocal Raman Microspectroscopy. *Photochem. Photobiol. Sci.* **2016**, *15*, 861–871.
- (40) Sator, P.-G.; Schmidt, J. B.; Hönigsmann, H. Comparison of Epidermal Hydration and Skin Surface Lipids in Healthy Individuals and in Patients with Atopic Dermatitis. *J. Am. Acad. Dermatol.* **2003**, *48*, 352–358.