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Comparison of Structure and organization of cutaneous lipids in a reconstructed skin model and human skin: spectroscopic imaging and chromatographic profiling

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Abstract:

The use of animals for scientific research is increasingly restricted by legislation, increasing the demand for human skin models. These constructs present comparable bulk lipid content to human skin. However, their permeability is significantly higher, limiting their applicability as models of barrier function, although the molecular origins of this reduced barrier function remain unclear.

This study analyses the Stratum Corneum (SC) of one such commercially available reconstructed skin model (RSM) compared to human SC by spectroscopic imaging and chromatographic profiling. Total lipid composition was compared by chromatographic analysis (HPLC). Raman spectroscopy was used to evaluate the conformational order, lateral packing and distribution of lipids in the surface and skin/RSM sections. Although HPLC indicates that all SC lipid classes are present, significant differences are observed in ceramide profiles. Raman imaging demonstrated that the RSM lipids are distributed in a non-continuous matrix, providing a better understanding of the limited barrier function.

Keywords: Human Skin, Artificial skin models, Stratum Corneum, Lipids, high performance liquid chromatography, Raman spectroscopy

Background:

EU and US legislation restrict the use of human and animal tissues (1,2). In Europe, the use of animal models for cosmetic research has been prohibited since 2009 (1,2) and the commercialization of cosmetic products tested on animals has been prohibited since 2013 (3). For these reasons, reconstructed human skin models (RSM) derived from cell cultures have recently been developed and are increasingly commercially available as *in vitro* alternatives to animal testing (4-9). Typical structures of such models are described in more detail in Supplementary Information.

Significant efforts have been devoted to the evaluation of the validity of RSMs substitutes for human skin. Comparison of the morphology at macroscopic and microscopic levels, analyses of biochemical markers, phototoxicity and irritancy testing have been performed (2, 7, 10-14). These constructs have, however, been demonstrated to be limited as models for human skin barrier function (1, 15-21), determined by composition and structure of lipids in the stratum corneum (SC) (22-25). However, no systematic studies of the structure and organization of cutaneous lipids in RSMs compared to human skin have been presented.

Questions Addressed

The purpose of the present study was to further the understanding of the barrier function of the SC in a commercially available *in vitro* RSM compared to human SC *ex vivo*.

The study addresses the equivalence of the global lipidic content of the SC and furthermore examines the structure and organisation of those lipids in the SC, laterally and in sections.

It demonstrates that although all SC lipids are present in the RSM, their heterogeneous distribution may be the cause of reduced barrier function in such models.

Experimental Design:

The study compared human abdominal skin obtained after plastic surgery with the RSM, Epiderm® (MatTek, USA). A comparison of the lipid profile was performed using High Performance Liquid Chromatography (HPLC). Raman spectroscopy was used to evaluate the conformational order and the lateral packing of SC lipids. Raman imaging was performed on the surface and sections of both the RSM and human skin to investigate the spatial distribution of the different structures.

Full details of Materials and Methods are provided in Supplemental Information

Results:

The lipid profiles of human skin and RSM showed similarities, as detailed in Supplementary Information, and previously observed by Ponec et al.(10). The comparison of the relative amounts (figure 1) shows that the RSM presents all major classes of lipids of human SC, although in different proportions. The main differences are observed in the composition of ceramides.

The use of Raman spectroscopy to examine the lateral packing and conformational order in SC lipids is well established (22, 26-30). The secondary structure and folding of keratin has similarly been assessed using Raman spectroscopy (51-56).

To examine the lipid barrier and secondary structure of the proteins present in the RSM compared to human samples, mean values (\pm standard deviations) were obtained for the frequency positioning and, where appropriate, FWHM of each of the Raman spectral features (table S1, Supplemental Information). Notably, the spectral analysis of the surface of the RSM showed 3 different spectral signatures (figure S2) obtained from distinct regions of the surface. Therefore, the lipid and protein descriptors derived from each zone are presented independently (table S1).

The analysis of the surface of the RSM showed a heterogeneous distribution of lipids. This was confirmed on a larger scale using spectral imaging combined with Non-negativity Constrained Least Squares fitting. Figure 2A shows the repartition of the keratin (A.1), cholesterol (A.2) and ceramides and fatty acids (A.3) on the surface of the RSM. Only the keratin is present in a continuous matrix at the surface. In contrast to human SC which presents a continuous matrix and homogenous distribution of ceramides, fatty acids and cholesterol (Figure S3), the cholesterol in the RSM is spatially separated and not mixed with ceramides and fatty acids, which are present largely as droplets at the surface.

The inhomogeneous lipid distribution and separation of different lipid classes at the SC surface prevents the formation of a continuous lipid barrier. This heterogeneous repartition may be the reason of the high permeability of RSMs compared to human SC.

To explore the global repartition of lipids and keratin underneath the surface, analysis of sections was performed. Reconstructed pseudo-color NCLS spectral images of the sections are presented in figure 2B.

In addition to the presence of keratin throughout the human SC sections (figure S3B.1), figures S3B.2 and S3B.3 show that cholesterol, ceramides and fatty acids present continuous homogenous matrices throughout the section, thus providing the barrier properties of the SC. Similar to the repartition observed at the surface of the artificial skin, figure 2B shows a non continuous lipid matrix across the SC section. Moreover, figure 2B.3 shows a deficiency of ceramides at depth with higher concentration at the surface.

Conclusion:

Although all human SC lipids are present in the selected RSM, the relative amounts differ significantly and the composition of ceramide showed important differences. These differences seem to have a direct impact on the formation of a continuous lipid matrix. Raman imaging demonstrated that the lipids in the model studied are present either in droplets (ceramides and fatty acids) or in separate zones (cholesterol) and thus do not form a continuous barrier. Although only one commercially available RSM has been studied, this may be the main reason for the reported relatively higher permeability of skin models in general, compared to human SC. Further studies are required to verify this and to explore, for example, whether the origin of the keratinocytes play a role in determining the structure and composition of the SC.

It should be noted, however, that the incubation process for the skin model is very different from that of the natural normal skin maturation, which is influenced by numerous endogenous and exogenous non controlled factors over a prolonged period of time. As a consequence, the sample to sample variability is significantly reduced compared to human samples and they represent reproducible standard models for proof of concept and inter-laboratory comparative studies of molecular activity, permeability and safety.

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Ali TFAYLI, Franck BONNIER, Hugh. J. BYRNE and Arlette BAILLET-GUFFROY designed the research study.

Zeineb FARHANE, Ali TFAYLI, Franck BONNIER, and Danielle LIBONG performed the research.

Ali TFAYLI, Franck BONNIER and Danielle LIBONG contributed essential reagents or tools

Ali TFAYLI, Franck BONNIER analysed the data

Ali TFAYLI, Franck BONNIER, Zeineb FARHANE, Hugh. J. BYRNE and Arlette BAILLET-GUFFROY wrote the paper

All authors confirm that:

- The data in the manuscript is original and the manuscript is not under consideration elsewhere;
- All authors have read and approved all versions of the manuscript, its content, and its submission to Experimental Dermatology;
- None of the manuscript contents have been previously published;
- No conflict of interest;

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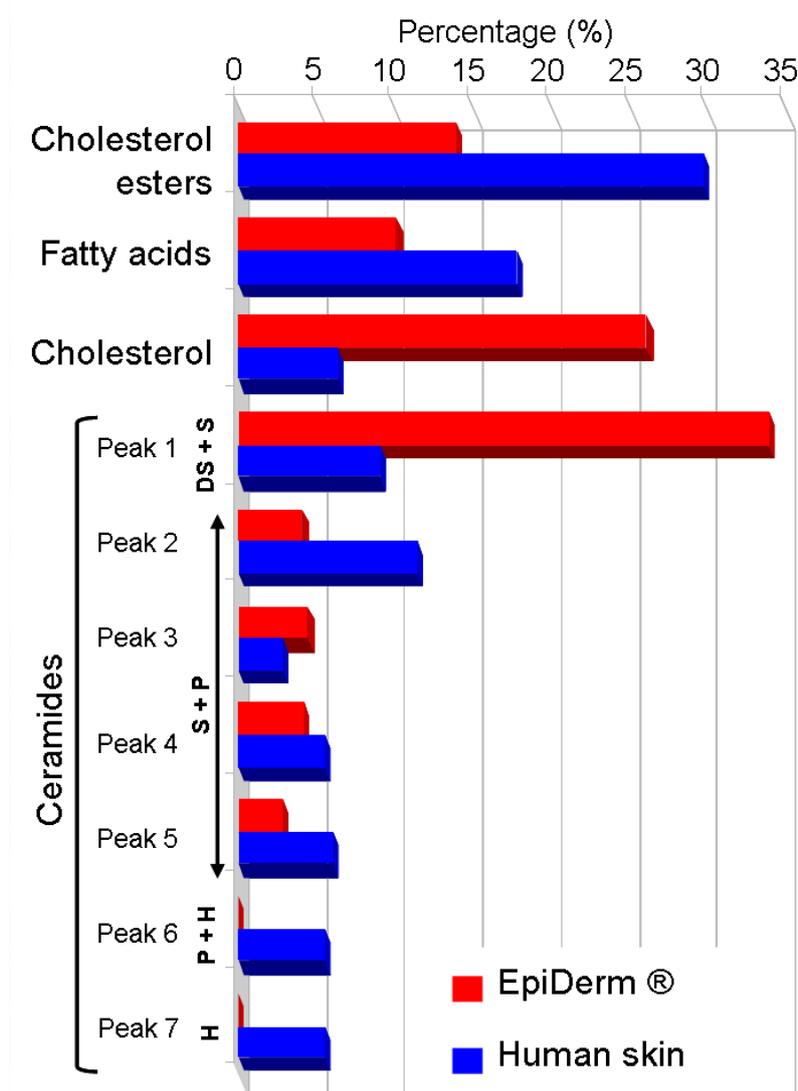


Figure 1: Relative amounts of lipids in the EpiDerm® model and human SC obtained from HPLC profiles. The ceramide region is composed of several peaks associated to the different subclasses (DS: dihydrosphingosine, S: sphingosine, P: phytosphingosine, H: hydroxysphingosine).

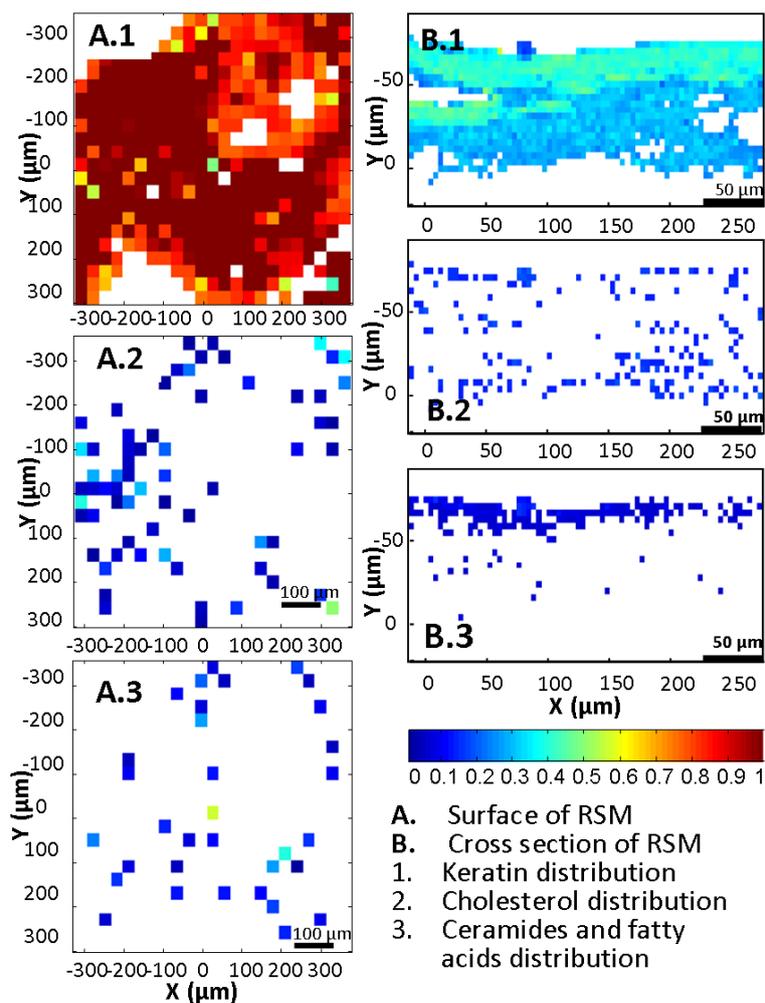


Figure 2: NCLS mapping on reconstructed skin model (RSM) representing the distribution of 1. keratin. 2: cholesterol. 3: ceramides and fatty acids. The color code represents the fraction of each molecule in each pixel of the image. A. Surface of the RSM. B. Section of the RSM.