Characterizing the structure of human skin substitutes by Infrared and Raman microspectroscopies

+[1,2,3]Leroy, M; [3]Auger, M; [1]Laroche, G and [2]Pouliot, R
+[1]Laboratoire d'Ingénierie de Surface (LIS), Centre de Recherche du Centre Hospitalier Universitaire de Québec (CRCHUQ), Département de génie des matériaux, Université Laval, Québec (QC), Canada; [2]Centre LOEX de l'Université Laval, Génie Tissulaire et Régénération: LOEX-Centre de Recherche du Centre Hospitalier Universitaire de Québec, Québec (QC), Canada; [3]Département de Chimie, PROTEO, CERMA, Université Laval, Québec (QC) Canada

Introduction

In vitro tests are prerequisite for the elaboration of new drug formulations. The development of new skin models closer to reality becomes essential to avoid tests on human, animal, or artificial membranes. With the advances in tissue engineering, it is now possible to produce human skin substitutes, consisting in a dermis and an epidermis, without any exogeneous material.[1] The skin acts mainly as a protective barrier from the external environment, thanks to the *stratum corneum* (SC) which is the outermost layer of the skin.[2] Vibrationnal spectroscopy enables the characterization of the organization of the different layers of the skin. In fact, spectral analyses of skin, by infrared and Raman spectroscopies, provide information on the in-depth order and conformation of the lipids chains, and secondary structure of proteins.[3]

Materials and Methods

This study was conducted in agreement with the Helsinki declaration and performed under the guidelines of the research ethics committee of the ‘‘Centre hospitalier affilié universitaire de Québec. Cells have been extracted from biopsies taken from breast reduction surgery.

Human skin substitutes have been prepared with cells extracted from biopsies using the self-assembly method developed by the LOEX.[4] Biopsies of these substitutes were then embedded in Optimum Cutting Temperature (OCT) gel and frozen for further analyses. For IR microspectroscopy, cryosections were placed on reflective-coated microscope slides. FTIR microscopy images were recorded with an Agilent 620 IR microscope equipped with a liquid-nitrogen cooled 32 x 32 focal plane array detector. Each of these 1024 detector elements measured 5.5 µm x 5.5 µm, which therefore corresponds to the pixel size in the infrared images. For Raman spectroscopy, cryosections were placed on microscope slides. Spectra were collected at few points in the SC, the living epidermis and the dermis using a LABRAM HR800 Raman spectrometer coupled to an Olympus BX fixed stage. An internal He-Ne laser set at 633 nm was used for the acquisition of spectra.

Results and discussion

The infrared spectra of skin substitutes exhibit features due to the stretching mode vibration of methyl and methylene groups in lipid acyl chains. The symmetric stretching mode vibration of the lipid methylene groups (2850 cm^{-1}) is sensitive to the acyl chain overall order. As can be seen in Figure 1, the lipid chain conformation exhibits drastic changes. Comparison of these data with histological analyses (not shown) demonstrates that these lipid order changes occur at the interface between the SC and the living epidermis. This evolution profile suggests that lipids in the SC are more ordered than those in the living epidermis (*trans* conformers versus *gauche* conformers). In addition, the amide I stretching vibration allowed determining that the proteins essentially adopted an α-helix conformation in the epidermis, probably associated with the presence of keratin, while typical spectrum of collagen was observed in the dermis (cells embedded in extracellular matrix).

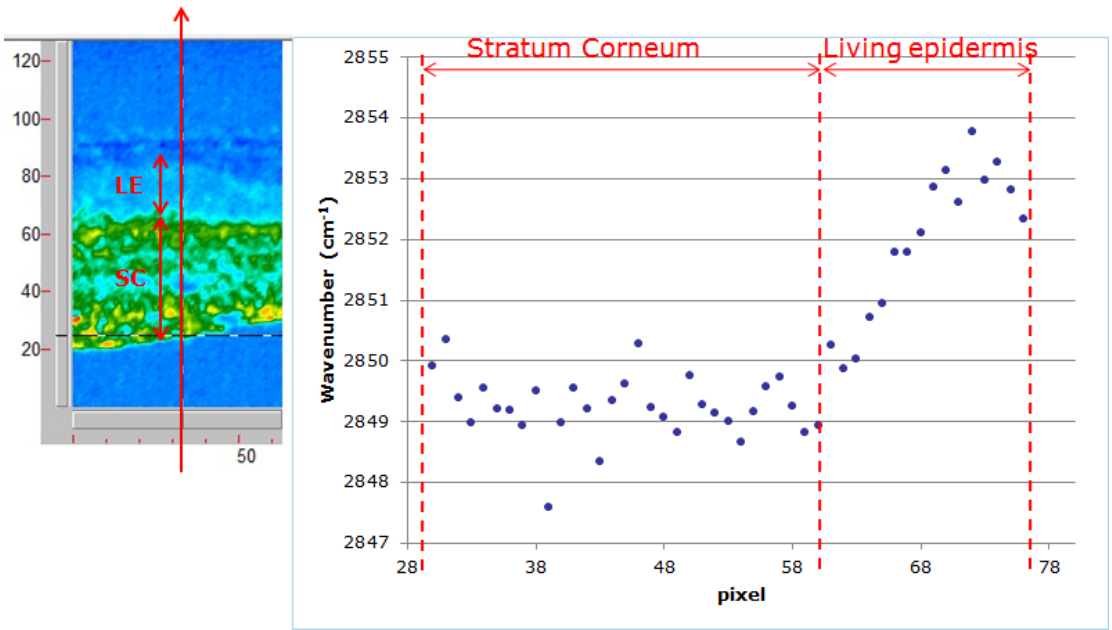

Figure 1 Evolution of the frequency of the symmetric CH_2 stretching mode as a function of the depth

In Raman microspectroscopy, the stretching –C-C modes at 1061 and 1128 cm^{-1} are characteristic of *trans* conformers while *gauche* conformers give rise to features at 1081 and 1100 cm^{-1}. The ratio of the intensities of the modes at 1061 cm^{-1} and at 1081 cm^{-1} shows that a higher proportion of *gauche* conformers is observed in the SC of the skin substitutes compared to Normal Human Skin (NHS). This suggests a decrease of the lipid matrix organization in the SC of the skin substitutes.

Conclusion

Infrared and Raman spectra of reconstructed skin taken from the epidermis and the dermis allowed characterizing the molecular organization of the equivalent layers. Vibrational microspectroscopies analyses show that the lipids are more ordered in the NHS than in the substitutes but the evolution profile of the organization of lipids as well as protein composition are similar.

Further experiments will take advantage of both IR and Raman microspectroscopies to identify the preferential pathways and evaluate the diffusion rate of exogenous molecules through human skin substitutes.

References

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