Abstract

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Title of the thesis: Corneocyte lipid envelope in vitro model development and validation I

The skin serves many functions, one of which is to defend the body and protect its internal systems. This defence is primarily carried out by the epidermis, specifically its outermost layer known as the stratum corneum (SC). The SC is composed of specialized cells called corneocytes that have undergone differentiation and developed an envelope directly beneath their cytoplasmatic membrane known as the corneocyte lipid envelope (CLE). This envelope is made up of lipids covalently bound to proteins on the corneocytes' surface and is believed to play a crucial role in supporting extracellular lamellae or promoting corneocyte cohesion. To gain a better understanding of its function, it is necessary to develop various models for studying the CLE.

The objective of this study was to establish and validate a method for isolating the CLE and to evaluate the effectiveness of CLE purification using gradient centrifugation. The initial step in the CLE isolation process involved extracting the SC from human skin, following previously published protocols, and extracting the free lipids from it. Subsequently, individual corneocytes needed to be separated from this delipidized SC in order to examine the isolated CLE. To purify this isolated fraction and remove any residual extraction buffers, gradient centrifugation with sucrose solutions was employed. However, due to significant losses and inadequate purification of the samples, various modifications were attempted during the purification process. Finally, after the CLE buffer extraction using sucrose solutions, the CLE fraction was purified from sucrose residues using water. The presence, purity, and residue content of the isolated CLE were confirmed using techniques such as optical microscopy, infrared spectroscopy, and dynamic light scattering.

In conclusion, the CLE was successfully isolated and purified using gradient centrifugation. However, given the time-consuming nature of this method and the significant losses encountered, it is recommendet to replace it with a simpler filtration method.