Abstract

Charles University, Faculty of Pharmacy in Hradec Králové

Department of Pharmaceutical Technology

Supervisor: PharmDr. Anna Paraskevopoulou, Ph.D.

Author: Petra Kašparová

Title of the thesis: Human skin explants as models for research of skin barrier functions

The skin protects the human organism from harmful environmental effects and maintains the stability of the internal environment of the human body. The protective function is considered the most important role of the skin. It is provided primarily by the uppermost layer of the epidermis, the stratum corneum (SC). The arrangement of the lipid layer surrounding keratinocytes is especially important for it. This is evidenced by the association between the increased incidence of skin diseases and the imbalance of present lipids.

This thesis deals with the development of an ex vivo model of human skin, which would be used in the future to investigate skin barrier and topically administered formulations. The aim of this thesis is to establish suitable ex vivo skin cultivation conditions to achieve its sufficient viability and at the same time the biosynthesis of skin lipids.

Initially, it was necessary to determine whether the adjustment of the skin graft thickness by dermatome is advantageous for their viability. Furthermore, the culture was maintained in four media containing DMEM and different supplements (Ham's F-12; fetal bovine serum, FBS). The effect of supplements addition on viability and lipid content in SC was investigated. Viability was quantified at defined days of cultivation by implementing the TTC assay. The lipid content of the SC was analyzed by HPTLC.

Due to the higher viability values obtained during the pilot study, the method was validated using dermatome-adjusted skin. The profitable presence of Ham's F-12 for long-term cultivation was confirmed. On the other hand, the role of the FBS could not be clearly proven. The main finding of this thesis is that using DMEM-F12 medium a viability comparable than the control is achieved. Concurrently, the increasing trend of the total lipid content in the SC was confirmed, indicating desirable growth of SC. To conclude, an ex vivo human skin model with preserved viability and increasing lipid content in the SC for 12 days of cultivation was developed and validated.