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23212 | Development of an *in vitro* skin cell model and comparison with *ex vivo* models: the case study of green cosmetic active ingredients

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Skin is the first physical barrier against pathogens and mechanical injuries, and stratum corneum, the outermost layer of the skin, is the primary barrier to therapeutic delivery. Skin is divided into the epidermis, which consists mainly of keratinocytes and the dermis, composed of fibroblasts, and extracellular matrix components. Skin models are crucial for assessing the safety and efficacy of cosmetic ingredients, especially under European Regulation (CE) No. 1223/2009 that bans animal testing in cosmetics [1]. While commercial skin models approved by the Organization for Economic Co-operation and Development (OECD) exist, they often lack physiological complexity, limiting their relevance for regulatory compliance [2]. With this work, we aim to develop and characterize a 3D *in vitro* skin model to evaluate the effect of green cosmetic active ingredients—catechin, epicatechin, chlorogenic acid, and neochlorogenic – due to their antioxidant and anti-aging properties. A keratinocyte (HaCaT)-fibroblast (HDF) co-culture and a hydrogel matrix were established to mimic native skin properties, with TEER measurements assessing barrier integrity and MTT assays evaluating cytotoxicity and viability. Permeability studies, performed using Franz cells and HPLC-MS analysis, compared compound penetration in the developed 3D model, *ex vivo* skin explants, and the commercial EpiSkin™ model. Preliminary results indicate that the developed 3D *in vitro* skin model successfully supports keratinocyte-fibroblast co-culture, with TEER values suggesting the establishment of a functional barrier. **Conclusion:** The development of a physiologically relevant 3D *in vitro* skin model represents a significant step toward improving *in vitro* testing of green cosmetic active ingredients. This work is an advancement on sustainable and ethical cosmetic testing, bridging the gap between traditional models and human physiology.

Keywords: European Regulation (CE) 1223/2009, *in vitro* skin models, *ex vivo* models, green active cosmetic ingredients, permeation studies.

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