# 3D skin *in vitro* model development for human skin wound healing testing

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The use of animal models for various compound testing has contributed to the knowledge of skin diseases, wound healing, and the development of new therapeutic options. However, human *in vitro* skin models, which include essential cellular and structural components would reduce animal experiments during the preclinical evaluation of novel therapeutic approaches.
The development of an HSC (human skin constructs) model is a challenge for many laboratories. HSCs are required for biomaterials, pharmaceuticals, cosmetics *in vitro* testing, and also for the development of complex skin wound therapeutics. The developed model should present a well-differentiated epidermis and dermis similar to the native human skin.

The aim of this work was to develop an HSC composed of all of the epidermis-dermis layers. Different 3D cell culture conditions were tested to optimize HSC maturation, using various combinations of component-free or fully defined media, and air-liquid interface (ALI) culture. Optimized culture conditions allowed the production of HSC by culturing human foreskin fibroblasts (HFFs) embedded in rat tail collagen I for 2–3 days in a fibroblast medium supplemented with FBS and antibiotics. After that culturing keratinocytes (HaCaTs) on top of the collagen for 3 days in a keratinocytes medium. Co-culture was then submerged overnight in a differentiation-formulated medium to stimulate cell-cell contact formation and finally placed at ALI for 15–20 days. Histological analysis revealed uniform distribution of HFFs in the dermal layer and their typical elongated morphology. The epidermal compartment showed a multi-layered differentiated structure.

The developed HSC represents a fundamental *in vitro* tool to evaluate biocompatibility
of biomaterials, safety, pharmacotoxicity, and effectiveness, as well as to investigate skin biology, skin disease pathogenesis, wound healing, and skin infection.

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