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## Comparison of explant culture and enzymatic digestion methods for human skin equivalents development

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Today, most studies of chemical hazard identification are conducted on animals. However, ethical issues raised by animal research have led to the development of human skin equivalents (HSEs) to evaluate, *in vitro*, skin toxicological responses. They are constructed with human skin cells and their toxicity screening capacity relies on the epidermis development, which depends on the donor and culture conditions of cells. In this study, two cells isolation methods (explant culture and enzymatic digestion) were compared. Skin samples were taken from three patients. Each sample was processed by such methods and divided into different groups: cryopreserved and non-cryopreserved cells obtained by enzymatic digestion and cultured *in vitro*, and cryopreserved and non-cryopreserved cells obtained by explant culture and cultured *in vitro*. HSEs were constructed by seeding keratinocytes on a fibrin gel containing fibroblasts. Barrier function was evaluated by performing a permeability assay and a lipid composition analysis. The morphology was analyzed by hematoxylin–eosin staining. For immunohistochemical epidermis characterization, different cytokeratins and other epidermal proteins were analyzed. It was possible to obtain HSEs showing similar morphological characteristics to those of native skin and expressing differentiation markers. Keratinocytes were able to differentiate producing different stratification layers and the presence of a barrier function was confirmed. In this study enzymatic digestion method allowed a faster growth of keratinocytes producing a healthier, thicker and more organized epidermis, suggesting that this method can provide a better tool to generate HSEs for toxicity screening.

### Biography

Catalina Gaviria A is a Bioengineer with a Master's degree in Biomedical Engineering from the Polytechnic University of Turin. Her experience has been focused on scientific research, and, currently, she is a member of the Tissue Engineering and Cell Therapy Group. As a PhD student, with a student loan from COLCIENCIAS (scholarship Program No.727 of 2015), she has been working on the development of human skin equivalents for toxicity screening project financed by COLCIENCIAS ("Convenio" FP44842-084-2015).

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