JOINT EVENT

11th International Conference on

Tissue Engineering & Regenerative Medicine

&

4th International Conference on **Synthetic Biology and Tissue Engineering**

October 18-20, 2018   Rome, Italy

Cytokine release in human skin equivalents cultures for the assessment of irritation

Catalina Gaviria A, Luz Marina Restrepo M and Natalia Becerra C
University of Antioquia, Colombia

Today, most studies of chemical hazard identification are conducted on animals. However, the 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 from human skin biopsies cells. Most of them consist of human keratinocytes deposited on a support and cultured in such a way as to form a stratified epidermis. In response to chemical and physical stimuli, keratinocytes produce and release proinflammatory cytokines which led to cutaneous inflammation, suggesting that measuring such responses could be an interesting tool to identify irritants and limit the misclassifications obtained with viability measurements. The aim of the present study is to determine the potential of cytokine release from HSEs as a predictive model for skin irritation. Skin cells were obtained from skin samples after informed consent and fibrin-based HSEs were constructed by seeding keratinocytes on fibroblast-containing fibrin gels. After 21 days of culture, the HSEs were used to classify different substances and were analyzed morphologically (hematoxylin-eosin staining). The assessment of irritation was based on proinflammatory cytokine release and cytotoxic measurements by MTT assay (according to 439 OECD tests guidelines). Skin models showing similar morphological characteristics to those of native skin were obtained. The combination of cytokine and viability assessments rather than individual measurements showed to be a better tool to classify irritants. The HSEs showed a high chemical classification capacity. Further studies should be done to produce correctly differentiated models in order to increase the prediction capacity.

catalinag.1989@gmail.com

Journal of Tissue Science & Engineering
ISSN: 2157-7552

Regenerative Medicine 2018 &
Synthetic Biology 2018
October 18-20, 2018

Volume 9

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