Evaluation of anti-inflammatory properties of cosmetic products and ingredients using *in vitro* human keratinocytes and reconstructed human epidermis

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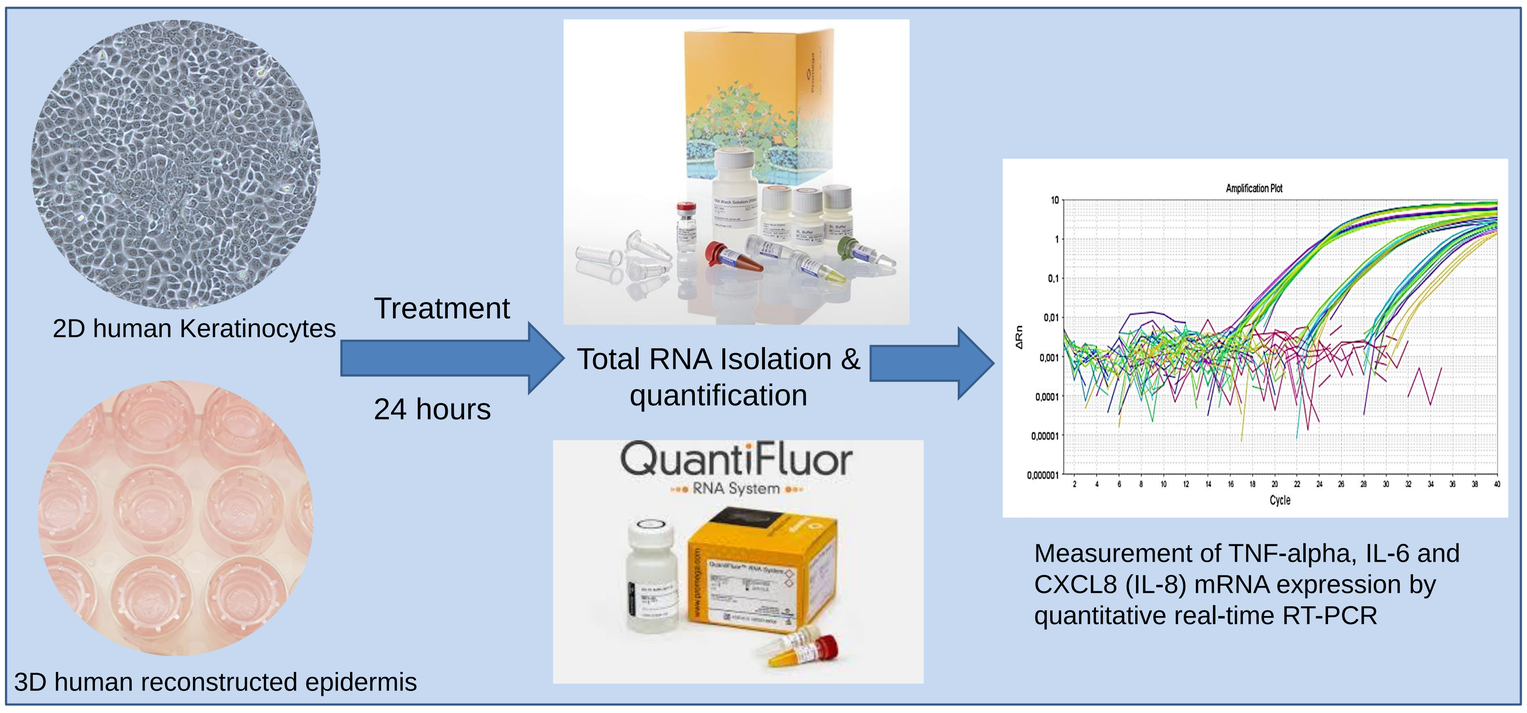
BACKGROUND

Atopic dermatitis is a typical inflammation-based disease that affects children and adults around the world. It is characterized by excessive skin dryness and itching, exfoliation, redness, and skin irritation. Anti-inflammatory agents are now commonly being incorporated into skin care products to combats inflammation by reducing the production of cytokines. With *in vitro* testing it is possible to evaluate the anti-inflammatory properties of cosmetic products and ingredients. The aim of this study was to compare the anti-inflammatory response of human keratinocytes in 2D cell culture and 3D human reconstructed epidermis.

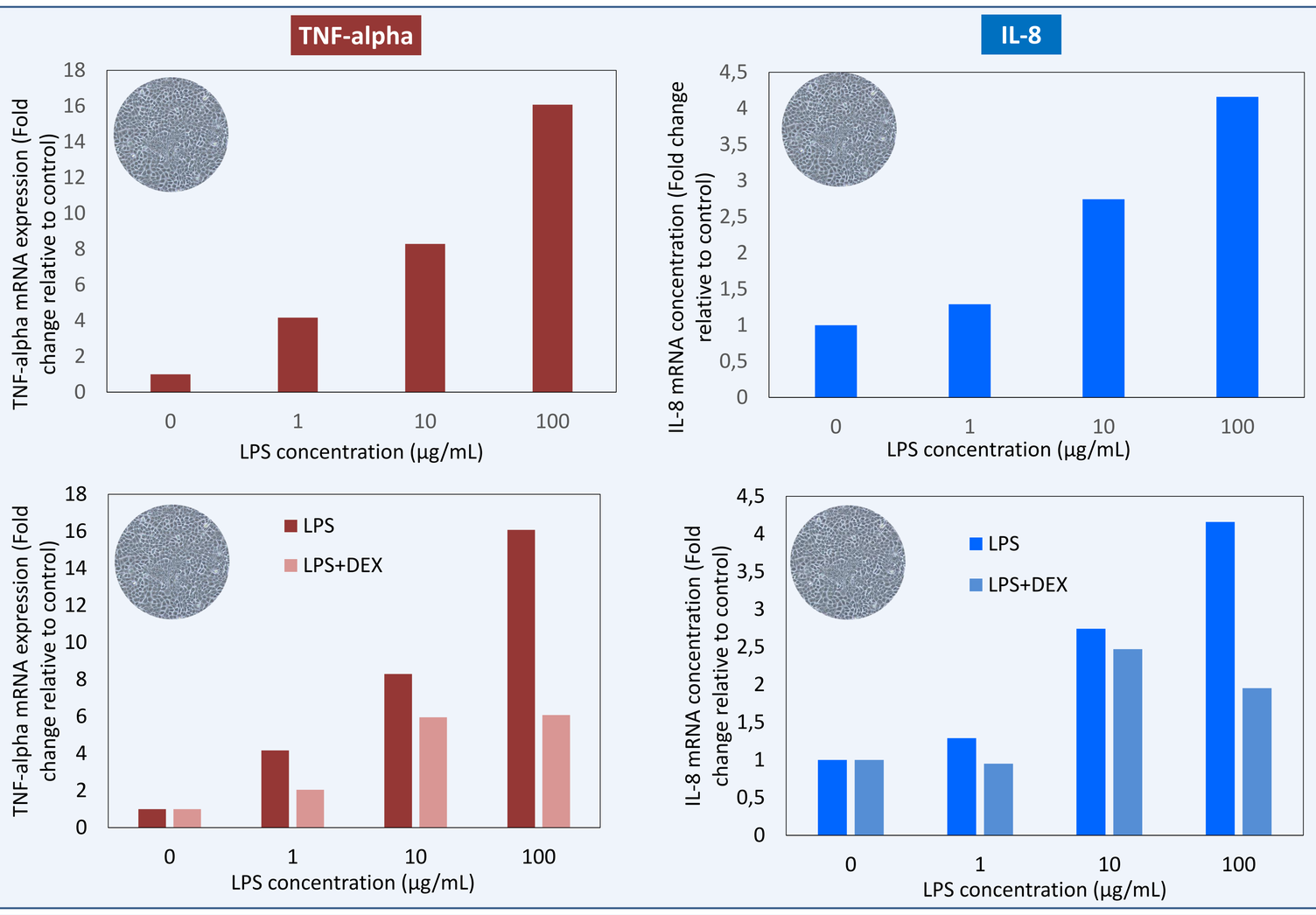
METHODS

Human keratinocytes in 2D cell culture and 3D human reconstructed epidermis (STERLAB, France) were treated for 24 hours with lipopolysaccharide (LPS) alone or with LPS and Dexamethasone. Untreated samples were used as control. Incubation was done at 37°C, 5% CO_2 and saturated humidity. After rinsing the samples at the end of treatment period, total RNA was isolated using Promega ReliaPrep™ RNA tissue miniprep system and quantified using QuantiFluor® kit (Promega France). mRNA expression of inflammation markers TNF-alpha, IL-6 and CXCL8 (IL-8) were measured by quantitative real time RT-PCR using TaqMan primers and probe (Life Technologies).

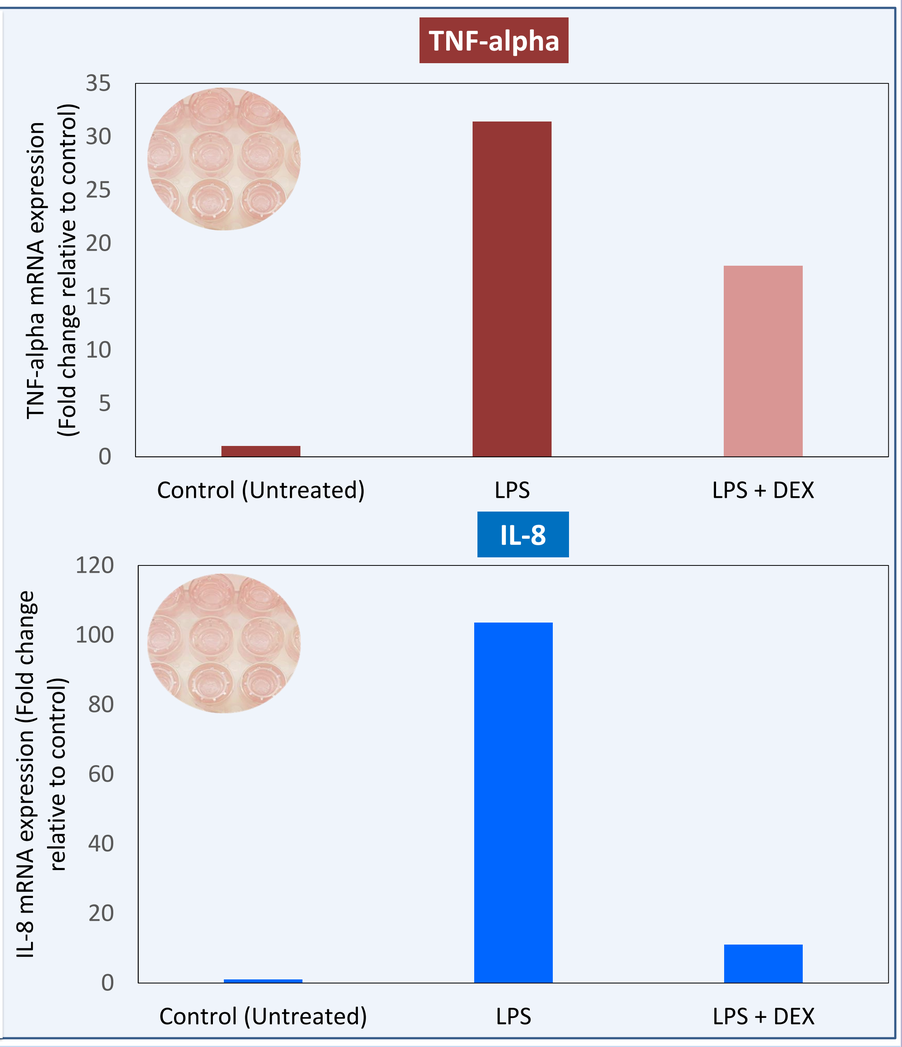


RESULTS

mRNA expression of TNF-alpha and IL-8 in 2D Human Keratinocytes



mRNA expression of TNF-alpha and IL-8 in 3D reconstructed human epidermis



- **In 2D human keratinocytes:**
  - LPS induced a concentration-dependent up regulation of mRNA expression of TNF-alpha and IL-8, confirming the inflammatory properties of LPS
  - Dexamethasone (100 µM) reduced the LPS induced gene expression of both inflammation markers, confirming the anti-inflammatory properties of Dexamethasone
- **In 3D human reconstructed epidermis:**
  - LPS treatment (100 µg/mL) up regulated the expression of TNF-alpha and IL-8 compared to control untreated
  - Dexamethasone (100 µM) reduced the LPS induced gene expression of TNF-alpha and IL-8

CONCLUSION

This study clearly shows that both 2D and 3D skin models are suitable to evaluate the anti-inflammatory properties of cosmetic products and ingredients. While 2D skin model can only be used with simple liquid formulations, 3D skin model can be used for liquid formulation and for even more complex formulations (cream, gel, unguent,…).

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