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Application Of reconstructed epidermis model in the evaluation of cosmetic soothing efficacy

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Introduction:

In recent years, consumers pay more and more attention to the efficacy of cosmetics, and the efficacy evaluation of the traditional cosmetics industry is facing new challenges[1].With the introduction of a large number of emerging technologies such as molecular biology, cell biology, and in vitro organ culture, in vitro detection has been gradually applied by the industry due to its advantages of short time-consuming, low cost and easy access to experimental materials. Studies have pointed out that the types and densities of cells contained in different layers of skin tissue are different, so in vitro cell experiments are not sufficient to accurately characterize the exact structure of human skin[2-4]. The reconstructed skin model that appeared in recent years is constructed in vitro by utilizing the structure of normal human skin cells, to obtain a complete three-dimensional anatomical structure, which can highly simulate human skin. The reconstructed skin model of the skin makes it very similar to the structure of normal human skin, which can replace the normal human skin to detect the effect of active substances or other stimuli on the expression of relevant essential proteins in the skin, and better reproduce the effect of cosmetics on human skin[5,6].Therefore, it can replace human skin to complete the safety detection and efficacy evaluation of cosmetic raw materials, and the efficacy evaluation of finished products, and is currently widely used in the cosmetics industry. In the evaluation of cosmetic efficacy, reconstructed skin model has become the development trend of the cosmetic industry. It is unclear whether the reconstructed skin model function similarly to the skin reactions of most people. To realize multi-dimensional unified scientific logic and ensure the validity of reconstructed skin model and the stability of the evaluation, further providing a scientific basis for the application of the reconstructed skin model in the evaluation of cosmetic soothing efficacy[7, 8].

Materials & Methods:

Materials

The RAW264.7 cells were obtained from the BeNa Culture Collection (BNCC,Beijing,China), Human epidermal growth medium and Skinovo-Ep human constructed epidermis model were purchased from Regenovo Biotechnology Co.,Ltd.(Hangzhou,China); The three basic skin care formulas with anti-inflammatory efficacy were selected as test samples.

Methods

Cell viability detection

RAW264.7 cells were treated at 37°C with different concentrations of sample for 24 h and then incubated at 37°C with 10 μl CCK-8 working solution for 1 h. The absorbance was detected using a Thermo Fisher Scientific Co., Ltd (Shanghai, China) at 450 nm.

Nitric oxide detection

RAW264.7 cells were treated at 37°C with LPS (1μg/ml) and different concentrations of sample treatment for 24h. NO production in the cell culture medium was determined using a Total Nitric Oxide Assay kit (Beyotime Institute of Biotechnology, Shanghai, China) according to the manufacturer's protocol.

Reverse transcription polymerase chain reaction (RT-PCR)

RAW264.7 cells were treated at 37°C with LPS (1μg/ml) and different concentrations of sample treatment for 24h,gene expressions of TNF-α,iNOS,IL-1β and IL-10 were determined with RT-PCR method. Intracellular total RNA was extracted from RAW264.7 cells using TaKaRa MiniBEST Universal RNA Extraction Kit, according to the manufacturer's instructions. The concentration and integrity of the RNA were measured at a 260/280 nm ratio. Then a PrimeScript II 1st Stand cDNA Synthesis Kit was used to synthesis cDNA, and TB Green® Fast qPCR Mix was used for fluorescence quantitative PCR operation. The GAPDH gene was used as invariant housekeeping gene internal control. The thermocycling conditions were as follows: 95°C for 3 min, followed by 45 cycles of 95°C for 15 sec, 55°C for 15 sec, 72°C for 15 sec, the last was 95°C for 15 sec, 60°C for 1 min, 95°C for 1sec. The relative gene expression was quantified by the comparative $2^{-\Delta\Delta CT}$ method.

Enzyme-linked immunosorbent assay (ELISA) analysis

The Skinovo-Epi epidermal model was randomly divided into a blank control group, SDS group and sample A-C group. Add 10 μL of 0.1% SDS to the upper surface of the model in the SDS group, add 10 μL 0.2% SDS to the upper surface of the sample group and add an equal volume mixture of the sample. After adding samples according to the experimental groups, the epidermal models were incubated in a 37°C incubator for 24 h, and IL-1α, IL-8 and PGE2 inflammatory mediator production in the cell culture medium were determined using a Fine Test ELISA Kit, according to the manufacturer's protocol.

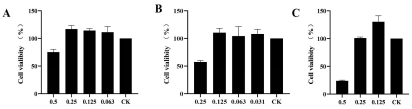
Statistical analysis

All data were presented as mean ± standard deviation, The above experiments were repeated 3 times. Statistical analyses were performed using Prism 8.3 software (GraphPad Software, Inc., La Jolla, CA, USA). The results were analyzed by one-way analysis of variance followed by a post-hoc Student's test for multiple comparisons.

Results & Discussion:

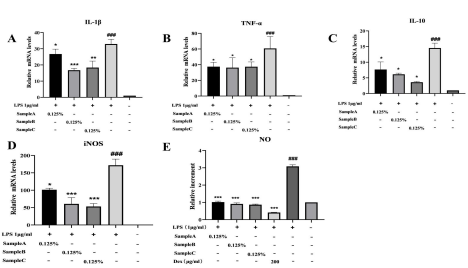
Effect of Three kinds of cosmetics on the viability of RAW264.7 cells

We examined the effect of three kinds of cosmetics on the viability of RAW264.7 cells. we screened the sample concentration with a cell survival rate of more than 90% as the safe concentration. the three sample concentrations were selected as 0.125%.



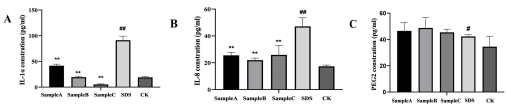
Three kinds of cosmetics inhibited the production of LPS-induced mRNA expressions of TNF-α,iNOS,IL-1β and IL-10 , secretion of Nitric oxide cytokines in the RAW264.7 cells

In order to evaluate the effect of cosmetics on proinflammatory mediators, gene expressions of TNF-α,iNOS,IL-1β and IL-10 were determined. Compared with the blank group, Figure showed that three kinds of cosmetics could markedly reduce the mRNAs expressions of TNF-α,iNOS,IL-1β and IL-10 (P<0.05) compared with the LPS alone-treated group. Besides, the three kinds of cosmetics has the ability to significantly inhibit NO production upregulated by LPS. These data indicated that three kinds of cosmetics decreased the expression level of LPS-induced pro-inflammatory mediators.



Three kinds of cosmetics inhibited the production of SDS-induced inflammatory factor expressions of IL-1α and IL-8 on the reconstructed epidermis model

ELISA Detection kit was used to examine the effect of three cosmetics on the production in the reconstructed epidermis model. Compared with the blank group, the concentrations of secreted IIL-1α,IL-8 and PGE2 inflammatory factors were increased significantly after SDS alone stimulation (P<0.05).Figure showed that three kinds of cosmetics could markedly reduce the inflammatory mediators expressions of IL-1α and IL-8 (P<0.05) compared with the SDS alone-treated group. But three kinds of cosmetics did not down-regulate the expression of PGE2 inflammatory factors. The data showed that Three kinds of cosmetics decreased the expression level of SDS-induced pro-inflammatory mediators.



Conclusions:

In this study, for three tested skin care formulas with anti-inflammatory properties, ELISA technique was employed to detect the expression of inflammatory factors in reconstructed epidermis model. And compared with the classical methods of cell biology, the scientificity and availability of reconstructed epidermis model in the evaluation of soothing effect were verified. It provides a new method and idea for the efficacy test of skin care formula in vitro.

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