A friendly strategy using reconstructed skin model to screen mild formulas

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Introduction:

As the ecological environment and climate change, the number of people with sensitive skin has increased.Therefore, to develop new test of selecting more mild formula for sensitive skin has been a new task for cosmetic industry.

In this study,we explore several concentration of SDS and found 0.3% SDS is a more appropriate concentration for setting up SDS treated barrier-damaged skin model.Further more,we develop a strategy (skin irritation test of normal skin model –HET-CAM test—SDS treated barrier-damaged skin model test) that can screen out more suitable formulas for sensitive skin and also the screening standard of this strategy can be used as an internal control of the enterprise.

Materials & Methods:

Reconstructed skin model to test skin irritation in vitro

The experiment used the reconstructed epidermal skin model(Episkin L2#,Episkin Shanghai) in accordance with OECD TG 439[1] and R. ROGUET[2] method.

HET-CAM test

the method is according to HET-CAM DB-ALM n° 96[3]. The transparent sample uses the irritation score method IS(B) (ICCVAM 2006) and the opaque sample uses the end point method (brantom1997[4]).

Reconstructed skin model experiment under SDS stimulation

Use different concentrations of 5%~0.05% SDS to treat for 15 minutes.After rinsing, continue to incubate for 18 hours, then add MTT 0.3mg/ml and incubate in a CO_2 incubator for 3 hours, then take the culture supernatant and freeze it for IL-1a testing. The model was taken out and added with acidic isopropanol, and the absorbance was measured at 570nm after 72h of extraction at 4°C. IL-1a elisa kits were purchase from Abcam.

SDS treated Barrier-damaged skin model test

Use 0.3% SDS to treat the skin model for 15 minutes, then rinse with PBS. After rinsing, add 150ul of each sample to spread evenly on the surface and continue to incubate for 18 hours, then rinse with PBS; the following operation is the same as above.

Results & Discussion:

1. Reconstructed skin model to test skin irritation in vitro

Table 1. Sample Information

Sample	Texture	Catergery
OM121121	Cream	CP
OM121131Z	Cream	CP
ON012232	Cream	CP
OM12311F	Emulsion	CP
M012141	Emulsion	OF
CMJ200616A	Cream	OF
M012211	Cream	OF

CP:Commercial Product； OF:Own Formula

- The commercially available products are form *Dr. Yu*, *WINQNA*, and *LA ROCHE-POSAY* brand all for sensitive skin.

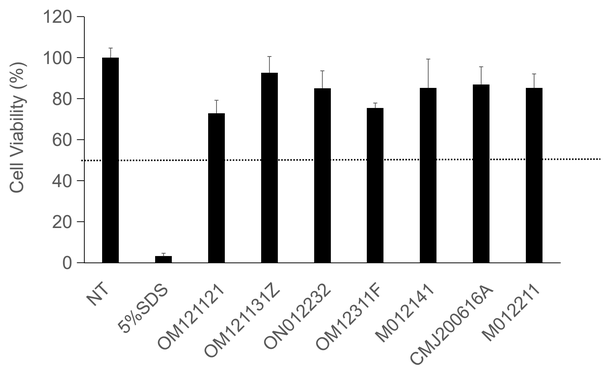


Fig. 1 Cell viability obtained from EpiSkin model exposed to samples

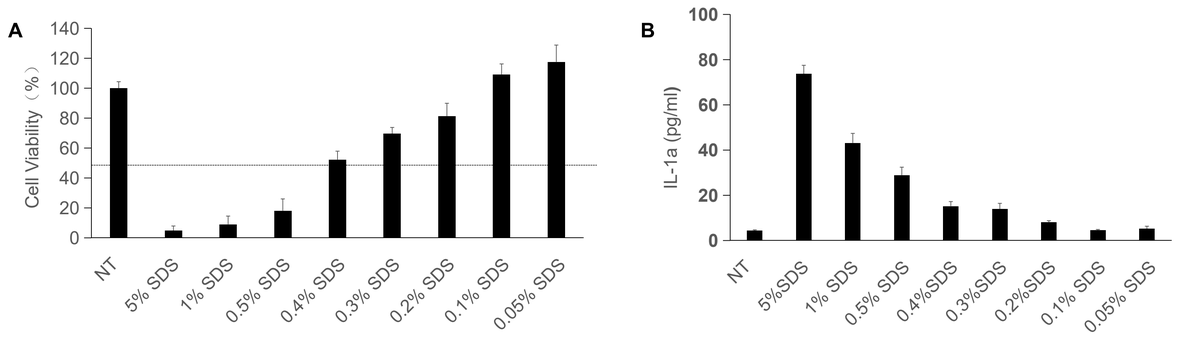
2. HET-CAM test

Table 2. HET-CAM Endpoint Score of samples

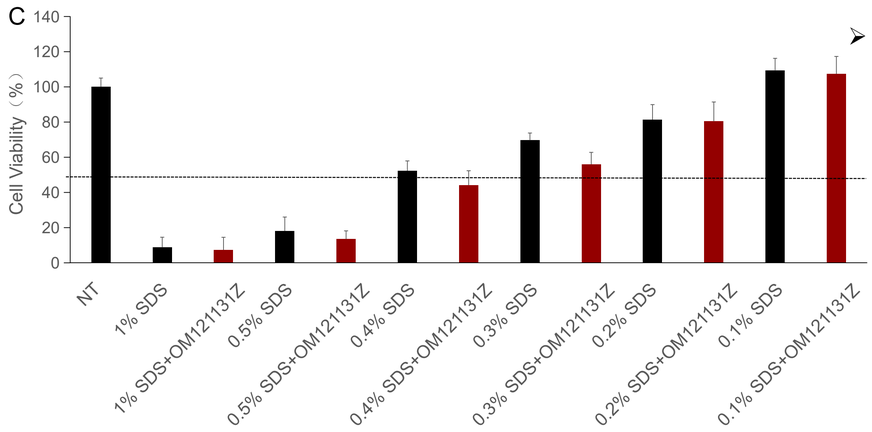
Sample	Concentration	HET-CAM S-Score (severity score)
OM121121	100%	6
OM121131Z	100%	6
ON012232	100%	8
OM12311F	100%	8
M012141	100%	7
CMJ200616A	100%	6
M012211	100%	8

- The results are shown in **Figure 1**. The cell viability of the test samples were all above 50%, and there was no skin irritation. The cell viability of the sample is between 70% and 95%.
- The scores of the tested emulsion and cream are all less than 12, concentrated in 6-8 as shown in **Table 2** . The irritation is relatively mild, and the scores are not have big difference.

3. Reconstructed skin model experiment under SDS stimulation



- Using 0.05% ~5%SDS to treat the skin model, the cell viability shows a decreasing trend, and the IL-a level shows an increasing trend (Figure 2 A, B).The cell viability of skin model treated with 0.1%SDS or below is close to NT, and the IL-1a content is also close to the NT level.The SDS treatment concentration that causes certain damage to the model and cell viability more than 50% is 0.4~0.2%.



- When we treat the model with a certain concentration of SDS and add the sample for 18 hours, if the cell viability is finally more than 50%, we take this as the standard for passing this SDS damage model. According to this principle, the concentration of 0.3% is selected here after using product OM121131Z for further verification (Figure 2C)

Fig. 2 A: Cell viability of the skin model treated with different concentration of SDS; B:IL-1a level of the skin model treated with different concentration of SDS.C: Cell viability of OM121131Z treated with different concentration of SDS

4. SDS treated Barrier-damaged skin model test

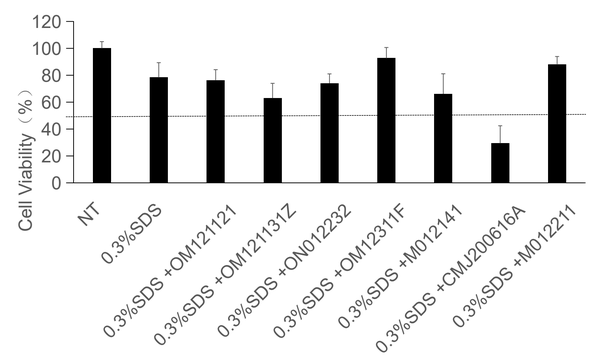


Fig. 3. Cell viability of each sample after 0.3% SDS treatment

- The cell viability of CMJ200616A in the 7 samples was less than 50% (Fig.3) which failed the SDS treated barrier-damaged skin model test, and the other 6 samples all passed the test witch may more suitable for sentisive skin. Moreover, the cell viability of the OM121131Z sample this time is still more than 50%, indicating that the model and this criterion have a certain degree of stability

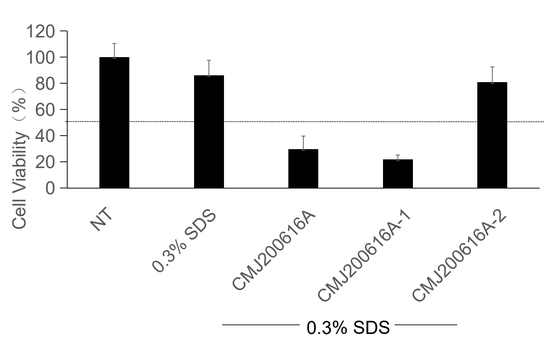


Fig. 4. Cell viability of adjusted CMJ200616A formula

- The CMJ200616A formula was optimized in two directions and then tested, and the cell viability of the CMJ200616A-2 was found >50% (Fig.4) passed the test. So SDS treated barrier-damaged skin model can not only screen out the formula for sensitive skin, but also help the formulator adjust the formula to provide consumers with a more gentle and suitable formula

Conclusions:

Under the premise of meeting requirements of the skin model skin irriation test at normal conditions (cell activity>50%), using HET-CAM test (HET-CAM score <12 points, non-transparent samples), and further screening of using SDS treated barrier-damaged skin model test is necessary to meet the needs of people with sensitive skin. This screening strategy can not only screen out more mild formulas, but also help formulators adjust and improve their formulas and conducive to screening out mild formulas for different needs.

References:

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