

## **Skin barrier repairing effect of Hymagic™-AcHA**

The skin barrier repairing effect of Sodium Acetylated Hyaluronate (Hymagic™-AcHA) was carried out using cells test and human test.

### **1. Promote epidermal cellular proliferation**

#### **1) Method**

This test was carried out using normal epidermal cells and damaged epidermal cells.

#### **2) Materials**

Cell strain: Human keratinocytes (HaCaT)

Sample: Hymagic-AcHA was diluted to different concentration (0.01/0.05%) using DEME culture medium (Hymagic-AcHA Batch no: 1610101). The samples were sterilized by a 0.22μm filter.

Reagent: Phenol, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), 5 mg/ml, in PBS, sterilized by a 0.22μm filter.

Apparatus: Inverted Microscope (OLYMPUS, CKX41), ELISA Reader (BIO-RAD, iMark).

#### **3) Operation**

(1) Inoculate human keratinocytes (HaCaT) on flat-bottomed 96-well culture plates at a density of  $5 \times 10^4$  cells/well, each 100μL, for 24h.

(2) Irradiate one of group cells by 7.2 J/cm<sup>2</sup> UVA as damaged cells group, the other as normal cells group and control group.

(3) Discard the medium, add 100μL Hymagic-AcHA solution sample to damaged cells group and normal cells group, and add 100μL DEME culture medium to control group. After inoculating for 48h, add 15μL of MTT solution to each well.

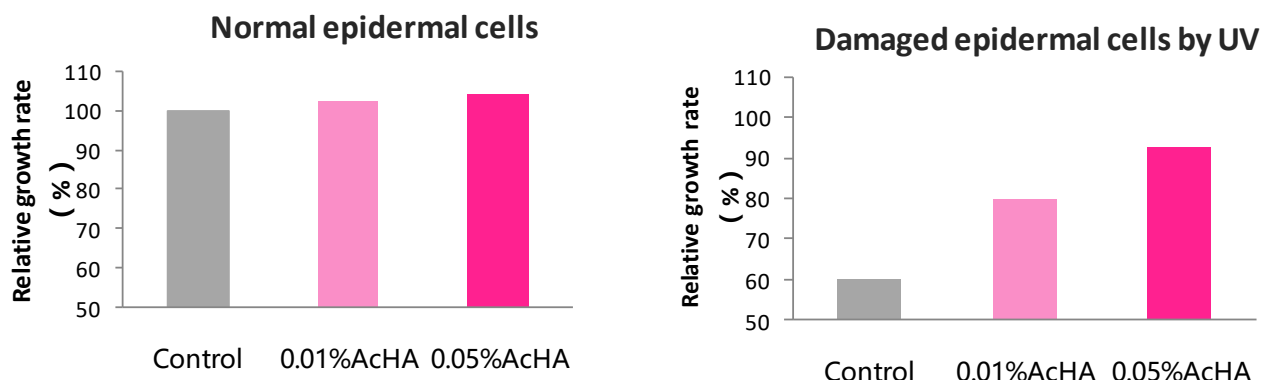
(4) Measure the optical density at 490 nm and calculate relative growth rate (RGR) using the following equation:

$$RGR = A / A_0 \times 100\%$$

Where  $A$  is the absorbance of test sample, and  $A_0$  is the absorbance of negative control.

#### 4) Results

Results show that, Hymagic-AcHA can promote cellular proliferation and repair damaged epidermal cells. And the higher concentration, the better repairing ability.



RGR(%)	Control	0.01%Hymagic-AcHA	0.05%Hymagic-AcHA
Normal cells	100	102.4	104.3
Damaged cells	60.1	79.6	92.4

## 2. Reduce water evaporation

### 1) Method

Test the skin TEWL of lotion containing Hymagic-AcHA in 12 hours.

### 2) Materials

Samples: The only difference between control and test samples was the lotion of test samples contained 0.1% Hymagic-AcHA or 0.1% HA.

Instruments: Tewameter TM300.

### 3) Volunteers

30 healthy women of 20~45 years old.

### 4. Operation

$3.0 \pm 0.1$  mg/cm<sup>2</sup> of control and test samples were respectively applied on the left and right forearm (4×4cm<sup>2</sup>). The skin TEWL of test area was measured before and after application of samples.

#### 5) Results

Results show that, after 2hrs application, the skin TEWL of Hymagic-AcHA group is reduced by 40.5%, compared with 26.9% of HA group and 12.6% of control group. And Hymagic-AcHA can reduce skin evaporation and last for 12 hours. Means that water evaporation reducing ability and skin barrier function of Hymagic-AcHA group is better than HA and control group.

Table 1. The comparison of skin TEWL

	Time	Control	0.1%HA	0.1% Hymagic-AcHA
TEWL (g/hm <sup>2</sup> )	0h	9.17	9.12	9.21
	2h	8.01	6.66	5.48
	4h	8.18	6.86	5.88
	6h	8.34	7.65	6.75
	8h	8.95	8.68	7.81
	10h	9.16	8.98	8.51
	12h	9.26	9.1	8.81

### 3. Conclusion

According to cells test and human test, Hymagic-AcHA can promote cellular proliferation and repair damaged epidermal cells, thus strengthen the barrier function of stratum corneum, and effectively reduce water evaporation from inside, improve the skin dryness and roughness.