# *Dendrobium officinale* polysaccharides promote fibroblasts migration and AQP3 expression

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HUIWEN BIOLOGY

## Introduction:

Although modern scientific studies have shown that *Dendrobium candidum* is rich in polysaccharides, and polysaccharides are also its main active components, which play an important role in enhancing immunity, anti-tumor and anti-oxidation . However, there are few reports on the effect of Dendrobium officinale polysaccharide on skin moisturizing and anti-aging. Therefore, in this study, the extracts containing DOP obtained by water extraction and alcohol precipitation were studied, and the migration rate of fibroblasts and the expression level of aquaporin AQP3 were used to study the moisturizing and anti-aging effect.

## Materials & Methods:

***Dendrobium officinale* Polysaccharide (DOP) extract**

Dendrobium officinale stems (100 g) were extracted in 2.5 L water for 2h at 80℃, Then red diatomite is used for coarse filtration and 0.25 micron filter membrane is used for fine filtration.Finally, alcohol was added to make the final concentration 65%, and the polysaccharide precipitated. The polysaccharide precipitated was collected and vacuum dried to obtain 10g for test on HS(68) cells.

**Fibroblast cell (HS68) culture**

The normal human newborn foreskin fibroblast cell line, HS68 cell (ATCC 1635), was obtained from (Life Science &Technology Co.,Ltd ). HS68 cells were plated in T-25 flask and grown in Dulbecco's modified Eagle's medium (DMEM , gibco) supplemented with 10% fetal bo-vine serum (FBS , gibco ) and 1% penicillin-streptomycin (Gibco) at 37°C with 5% CO2 atmosphere.

**Measurement of migration rate of fibroblasts**

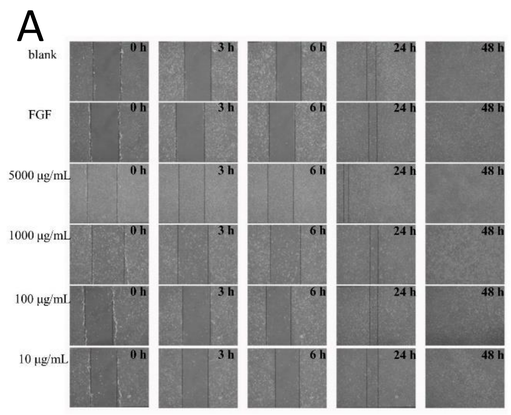
HS68 cells growing to about 80% in the culture dish were recycled into the centrifuge tube on the aseptic operation table, counted by cell counting instrument, and inoculated into the 6-well cell culture plate according to 7.0 ~ 8.0X105cells/Well. The head of the sterilizing pipette was used to scratch the center of the monolayer cells at the bottom of the 6-well plate perpendicular to the marking line, flush the floating cells with PBS solution, add drugs according to the experimental design, and put them into an incubator overnight. The samples to be tested were configured with corresponding concentration, and then filtered and sterilized by 0.22μm membrane. Cell scratch test grouping: sample concentration setting: DOP concentration 5000 μg/mL, 1000 μg/mL, 100 μg/mL, 10 μg/mL, negative control: blank (without sample), positive control: EGF (10 ng/mL). HS68 cells were cultured in an incubator at 37 ℃ with target concentration of DOP. The migration characteristics of cells were observed by taking photos under microscope at 0, 3, 6, 24 and 48 h.

**Extraction of total RNA and cDNA synthesis**

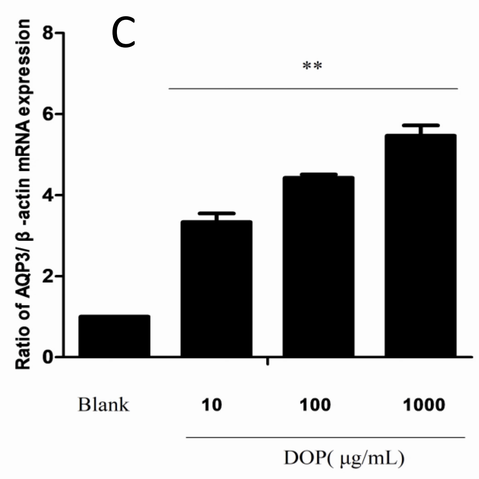
HS68 cells fused to about 80% were inoculated in a 6-well plate and cultured in a 37℃, 5% CO2 incubator for 24 h. DOP was divided into three dose groups (1000 μg/mL, 100 μg/mL, 10 μg/mL), and a blank control group was set. The total RNA was extracted by using TriZol (TAKARA) following the manufacturer's protocol. RNA con-centrations were determined using Qubit® RNA Assay kit (TAKARA). High Capacity RNA tocDNA kit (TAKARA) was then used for cDNA synthesis following the manufacturer's protocol.The cDNA was stored at −20°C until use. Using Power SYBR Green PCR Mix (Invitrogen) following the manufacturer's pro-tocol, qRT-PCR reactions were performed in tripli-cates. Primers for the amplification of AQP3 (F: 5'-GTTCATAGGCACAGCCTCCC-3', R:5'-GCAAGGGCTGTAAAAAGGCG-3 ' ), β-actin(F: 5 '-ATGTGGATCAGCAAGCAGGA-3 ' , R: 5 '-AAGGGTGTAAAACGCAGCTCA-3 ' ) were purchased from Sangon Biotec. β-actin was used as an endogenous control gene. qRT-PCR reactions were performed on a CFX -Connect Real Time PCR System(Bio-red). The mRNA expression levels of Aquaporin (AQP3) was evaluated relative to the levels of β-actin.

## Results & Discussion:

A




C




B

| Group | Concentration | Migration distance of HS68 cells（pixel） | | | |
|---|---|---|---|---|---|
| | | 3 h | 6 h | 24 h | 48 h |
| Blank | | 18.00±2.35 | 15.00±11.33 | 212.51±16.49 | 538.00±32.38 |
| EGF | 10 ng/mL | 17.13±4.47 | 19.00±13.26 | 257.00±17.12 | 556.73±30.19 |
| DOP | 5000 μg/mL | 49.67±4.14 | 59.20±13.31 | 193.45±18.61 | 689.46±28.09 |
| | 1000 μg/mL | 64.27±3.59 | 138.13±15.10 | 347.56±19.74 | 587.36±35.07 |
| | 100 μg/mL | 10.00±2.17 | 44.15±10.77 | 264.03±19.00 | 580.00±34.79 |
| | 10 μg/mL | 18.37±3.19 | 72.35±12.81 | 219.09±17.23 | 544.19±31.99 |

Fig A,B. Effect of DOP on migration characteristics of fibroblast HS68 cells in different time periods.

Fig C . Effect of DOP on AQP3 expression in fibroblast HS68 cells.

**Discussion**

In this study, we examined the possible effects of DOP as a natural compound for cosmetics through diverse assays in vitro. Several in vitro assay results showed the strong moisturizing and anti-aging activity of DOP treatment. Interestingly, the effect of DOP as moisturizing and anti-aging were correlated with the concentration of DOP. The anti-aging activity of DOP (1000 μg/mL) was comparable to that of EGF (10 ng/mL) or much higher than blank. Chen et al. (2015) in vivo experiments show that Dendrobium officinale polysaccharide can quickly moisturize almost as well as hyaluronic acid . Our experiment showed that DOP could realize the moisturizing effect by promoting the expression of AQP3. At the same time, with the increase of concentration, the promoting effect on AQP3 is more obvious. This also explains why DOP can so quick to moisturize.

## Conclusions:

Here, we evaluated DOP as a macromolecular polysaccharide have moisturizing and anti-aging funcation through several in vitro. On the one hand, the results showed that DOP could accelerate the cell migration by promoting the proliferation of fibroblasts to achieve the purpose of anti-aging, and on the other hand, it could achieve the purpose of moisturizing by promoting the expression of AQP3.SO our study demonstrated that DOP is an agent for anti-aging cosmetics ingredient

## Aknowledgments:

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## References:

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