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The bioactive peptide extracts from *Euglena gracilis*——a novel cosmetic ingredient as safeguard to eczema and diaper rash

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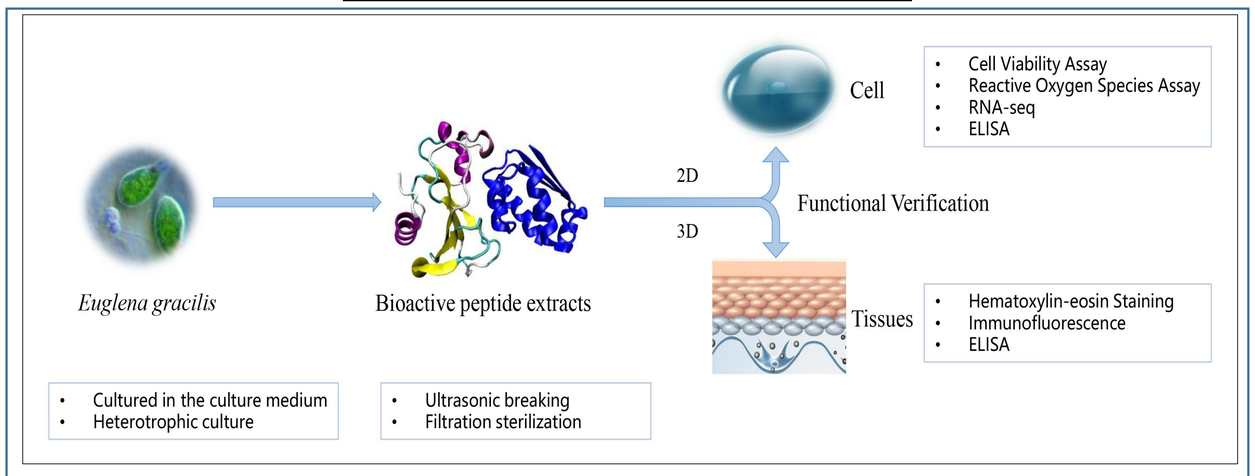
Introduction:

Euglena gracili, belonging to algae, is a group of free-living, single-celled flagellates with high protein and amino acid content, living in the aquatic environments [1]. And the surface is covered with an elastic pellicle with a diagonal pattern. *Euglena gracil* has a long evolutionary history and has wide-range adaptability to various environments, demonstrating the potential to apply in health supplements as well as cosmeceuticals by extracting active ingredients from it.

To explore the potential function of algae proteins, some research found that algae proteins were provided with multiple function on skin whitening, anti-aging, anticancer, antioxidant, anti-inflammation, and antimicrobial agents, especially on cosmeceutical potentials and applications[2-3].

For relieve or further cure eczema and diaper rash in infants, we examine the possible protective function of the bioactive peptide (a small molecular weight fraction of protein) extracts from *Euglena gracilis* (BPE), combining a variety of biological experiments on 2D skin cell models and 3D skin models, to validate the putative anti-eczema or anti-diaper rash activity of BPE in infant's skin and to screen other potential functions that may exist.

Materials & Methods:



Euglena gracilis was cultured in the culture medium by heterotrophic culture (with controlled light exposure). After the exponential growth, *Euglena gracilis* cells were collected, then ultrasonic breaking and filtration sterilization were used to get crude extract of *Euglena gracilis* cell. Finally, the crude extract was hydrolyzed by neutral protease to get bioactive peptide fraction.

The functional study of bioactive peptide extracts(BPE) from *Euglena gracilis* was evaluated by Cell Viability Assay, Reactive Oxygen Species Assay, RNA-seq and ELISA in 2D cell models. Meanwhile, in 3D skin models, Hematoxylin-eosin Staining, Immunofluorescence and ELISA were used to evaluate the function of bioactive peptide extracts(BPE).

Conclusions:

As common infant skin problems, eczema and diaper rash usually make infants suffer and problematic to their parents. The research found that the bioactive peptide fraction extracts from an ancient Algea called *Euglena gracili* shows the ideal function of anti-inflammatory, barrier-repairing and antioxidant activity, which provides a new path to prevent eczema and diaper rash in infant populations.

Aknowledgments:

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Results & Discussion:

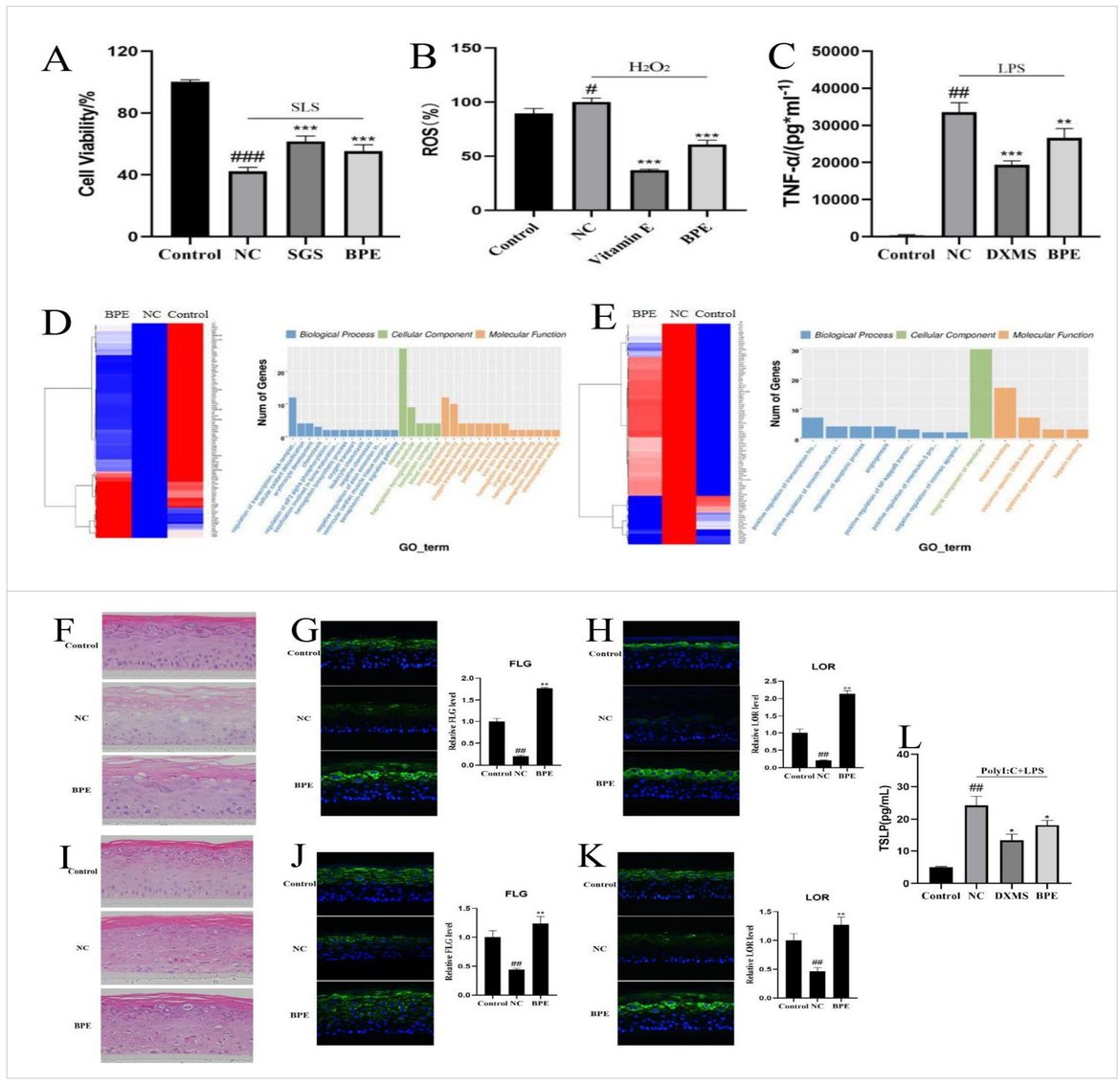

Figure. The functional discovery of bioactive peptide extracts(BPE) from *Euglena gracilis* for cosmetic products. In 2D cell models, the cell viability(A) of HaCaT cell was evaluated. The effect of BPE on ROS(B) and TNF-α(C) level were detected on HDF and RAW264.7 cells. The heatmap and GO enrichment analysis of genes upregulated(D) and downregulated(E) by BPE was shown. In 3D skin models, the specific 3D model recapture diaper rash was established, the effect of BPE on 3D model(F), FLG(G) and LOR(H) level were detected by HE staining and immunofluorescence staining. Besides, the specific 3D model recapture eczema was also established, the effect of BPE on 3D model(I), FLG(J), LOR(K) and TSLP(L) level were detected by HE staining, immunofluorescence staining and ELISA. NC: negative control; SGS: anti-inflammatory drugs for adults, DXMS: dexamethasone. Significant differences were determined by t-test; *p<0.05, **p<0.01, ***p<0.001, compared with NC; #p<0.05, ##p<0.01, ###p<0.001, compared with the control.

In 2D skin cell models, HaCaT cells, induced by SLS, were incubated with BPE for 24 hours and the cell viability was analyzed by CCK-8. As showed in Fig.(A), BPE was able to reduce a loss of cell viability caused by SLS, showing an effective barrier-repairing function on HaCaT cells. Meanwhile, as an important indicator of skin aging, the ROS level was detected on HDF cells. Induced by H_2O_2 for 2 hours, BPE performed a potential function of antioxidant by downregulating the ROS level (Fig. B). Besides, after induced by LPS for 24 hours, the TNF-α level of RAW 264.7 cell was notably upregulated while BPE could significantly decrease the TNF-α level (Fig. C). As shown in Fig. D-E, 107 genes were upregulated and 105 target genes were downregulated under the function of BPE, the candidate target genes were further analyzed by Gene Ontology analysis and GO enrichment analysis, which suggested that BPE has a potential anti-inflammation function.

To further study the effect of BPE, the 3D skin model, Epikutis®, which is closer to the skin characteristics of infants, was chosen to examine the function of BPE. The above results evidenced that BPE could significantly ameliorated Epikutis®'s damage induced by SLS, Poly I:C and LPS, indicating that BPE has a barrier-repairing function on eczema and diaper rash(Fig. F, I). .Furthermore, the FLG and LOR protein were detected by immunofluorescence (Fig. G-K). As important molecules that connects keratin fibers, FLG and LOR protein play an important role in the process of barrier-repairing in the stratum corneum of human skin [4-5]. The fluorescence result suggested that BPE could upregulated the level of FLG and LOR in eczema and diaper rash. In addition, TSLP, as a key signaling molecule during the process of eczema, was also detected by ELISA [6]. In view of this, we found that BPE had a reducing effect on the TSLP level, proving that BPE could obviously improve the inflammation response of eczema(Fig. L).

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