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# *"Research on the Enhancement of the Flexibility of Oat β - Glucan by Physical Methods and Its Repair and Anti - aging Efficacy"*

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## 1. Introduction

β-(1→3, 1→4)-glucan in oats, commonly known as oat β-glucan(OG), is a non-starch polysaccharide located in the cell walls of oat endosperm and aleurone layers.. In recent years, oat β-glucan has gained extensive application in the cosmetics industry, thanks to its remarkable functions in moisturization, repair, soothing, and anti-aging[1]. However, for polysaccharides like oat β-glucan, the instability of biological activity, stemming from the structural diversity and uncertainty, remains a critical bottleneck in their development. Current research predominantly focuses on the impact of molecular weight on functionality, while the study of solution conformations remains relatively scarce.
High-pressure microfluidization (HPMF) is an advanced technique that employs high pressure and microjet technology for precise material processing. Studies indicate that HPMF treatment does not alter the molecular structure of oat β-glucan but achieves modification by changing its molecular conformation or the entanglement of molecular chains[2]. Therefore, HPMF presents a viable approach for flexibly modifying the structure of oat β-glucan.
Flexible polysaccharides represent a highly active conformation of polysaccharides, which can be obtained through chemical or physical modification. Currently, the predominant modification methods primarily involve chemical approaches, such as sulfation and phosphorylation[3], while physical modification methods that preserve the primary structure of polysaccharides remain understudied. Physical modification, however, offers a distinct advantage: it enhances polysaccharide flexibility and activity without altering the primary structure, thereby ensuring raw material safety. Consequently, there is a pressing need to explore physical modification methods for OG and investigate how solution conformations influence its skincare activity. This study aims to elucidate the effects of HPMF on the structure and efficacy of oat β-glucan.

## 2. Materials and Methods

### *2.1 Instruments*

**Table 1. List of Experimental Instruments**

| Equipment | Type | Equipment | Type |
|---|---|---|---|

| | | | |
|---|---|---|---|
| High-performance liquid chromatograph (HPLC) | Ion chromatography | Infrared spectrometer | Evaskin |
| Differential refractive index detector | Atomic force microscope (AFM) | Inverted fluorescence microscope | VISIA CR |
| Laser light scattering detector | Carbon Dioxide Laser Therapeutic Apparatus | Cell incubator | Skin ultrasound |
| Nitrogen purging instrument | Scanning electron microscope (SEM) | Skin elasticity tester | High-pressure Microfluidic Homogenizer |

*2.2. paration of Oat β – Glucan(OG) and Flexible Oat β – Glucan(FOG)*

Starting with oat bran, hot - water extraction yields the OG crude extract solution. Ethanol precipitation is then carried out on this solution to form OG ethanol precipitation floc, which is redissolved in hot water to create a 1% OG solution. The 1% OG solution can be freeze - dried to obtain OG freeze - dried powder. Additionally, the 1% OG solution undergoes HPMF at 120 Mpa to form a 1% FOG solution, which is subsequently freeze - dried to get FOG freeze - dried powder.

*2.3.termination of Molecular Weight and Molecular Configuration*

OG and FOG were dissolved in 0.1M NaNO3 aqueous solution containing 0.02% NaN3 at the concentration of 1 mg/mL and filtered through a filter of 0.45 μm pore size. Refer to the method of Zheng[4] and use High - Performance Size Exclusion Chromatography (HPSEC) to determine the molecular weights and molecular configurations of OG and FOG.

*2.4.etection of Monosaccharide Composition*

Approximately 5 mg of OG or FOG was hydrolyzed with trifluoroacetic acid (2 M) at 121 °C for 2 h in a sealed tube. Dry the sample with nitrogen. Add methanol to wash, then blow dry, repeat methanol wash 2-3 times. The residue was re-dissolved in deionized water and filtered through 0.22 μm microporous filtering film for measurement. The detection was carried out using High - Performance Anion - Exchange Chromatography (HPAEC)[5], and monosaccharide standards were used for calibration.

*2.6. Morphological detection of FOG and OG*

The lyophilized powders of OG and FOG were adhered to the surface of the conductive adhesive. After sputtering with gold, the morphology was observed and photographed under a scanning electron microscope. Prepare OG and FOG solutions with a concentration of 1 mg/mL respectively, then drop them onto the mica sheet. And then, use the Magnetic AC (MAC) mode for atomic force microscopy imaging and recording.Grind and press OG and FOG respectively with potassium bromide into tablets, and then use FTIR to conduct infrared spectrum scanning[6].

*2.8. Cell culture and ELISA*

HFF 1 cells and HaCaT cells were cultured in DMEM containing 10% FBS and penicillin-streptomycin in an environment with 5% carbon dioxide at 37 °C and humid air. The ELISA method was used to detect the content of type III collagen in HFF 1 cells after 24 - hour treatment with OG or FOG.

*2.9.Wound healing assay*

The experimental method of the wound healing assay was slightly modified based on reference[7].

*2.10.Experimental Study of Fractional Laser Treatment on Human Face*

The skin damage and recovery model was designed to investigate the repair potential of OG and FOG on the skin barrier. Skin barrier damage was induced on the flexor side of volunteers'

forearms by using a $CO_2$ fractional laser device conducted by licensed doctors from Beijing Zell Cosmetic Clinic.Ten volunteers (aged 20–35 years old) were selected according to the inclusion and exclusion criteria. After the fractional laser treatment, use the samples according to the following Table 2. Then, use the VISIA CR and skin ultrasound for imaging, and use a skin elasticity tester to detect the skin elasticity.

**Table 2.** Protocol for Human Trials of Fractional Laser

| Treatment | CO2 Ablative Fractional Laser(70mJ/cm$^2$) | | |
|---|---|---|---|
| Acute Phase and Recovery Phase (Post-Treatment Days 1-7) | 0.05% FOG Serum | Serum Placebo | 5000IU/mL EGF Serum |
| Remodeling Phase (Post-Treatment Days 8-28) | 0.05% FOG Cream | Cream Placebo | 0.03% collagen III Cream |

*2.11.Evaluation by People with Sensitive Skin*

Thirty - three volunteers with sensitive skin were recruited. After using the 0.05% FOG cream for 14 and 28 days, EVASKIN, skin ultrasound, skin elasticity tester, and water dispersion tester were used for detection respectively.

*2.12. Statistical analysis*

All figures were generated using GraphPad Prism 8.0 unless otherwise specified. All experiments were performed at least three times, and representative data were presented. The statistical differences ($p<0.05$) among multiple groups ($\geqslant 3$) were calculated using one-way ANOVA. Statistical analysis was performed using GraphPad Prism software.

## 3. Results

*3.1. Characterization of the basic structures of OG and FOG*

Oat β-glucan, a polymer, is formed by the linkage of monomeric β-D-glucopyranose units through β-(1→3) and β-(1→4) glycosidic bonds. Its molecular weights can vary significantly, spanning from $3\times10^3$ Da to $5\times10^6$ Da[8], primarily due to differences in oat varieties and extraction methodologies.To elucidate the characteristics of oat β-glucan, the molecular weights of OG and FOG were determined using HPSEC, while their monosaccharide compositions were analyzed via HPAEC. As presented in Table 3, the molecular weights of OG and FOG were found to be $4.9\times10^4$ Da and $4.7\times10^4$ Da, respectively. Both OG and FOG shared a monosaccharide composition consisting of Glucose, Rhamnose, and Xylose, with glucose accounting for over 94% of the total. These results demonstrate that HPMF treatment exerts no significant influence on either the molecular weight or the monosaccharide composition of oat β-glucan.

**Table 3.** The molecular weights and monosaccharide compositions of OG and FOG

| | Molecular weight parameter | | | | | Monosaccharide composition | | |
|---|---|---|---|---|---|---|---|---|
| | Mn (kDa) | Mp (kDa) | Mw (kDa) | Mz (kDa) | Polydispersity (Mw/Mn) | Rha,% | Glc,% | Xyl,% |
| FOG | 31.880 | 37.462 | 46.913 | 66.587 | 1.472 | 2.35% | 95.36% | 2.29% |
| OG | 33.227 | 38.338 | 48.577 | 70.740 | 1.462 | 2.61% | 94.30% | 3.09% |

To further investigate the impact of HPMF treatment on the structural integrity of oat β-glucan, Fourier-transform infrared spectroscopy (FTIR) was employed to examine the functional group changes in OG and FOG (Figure 1). In the FTIR spectra of these samples, characteristic absorption peaks typical of polysaccharides were observed. Notably, the infrared

spectra of OG and FOG displayed essentially identical structures, which proves that the HPMF treatment has little effect on the primary structure of oat β-glucan.

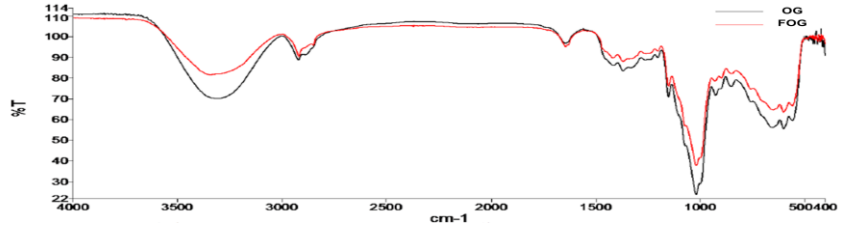
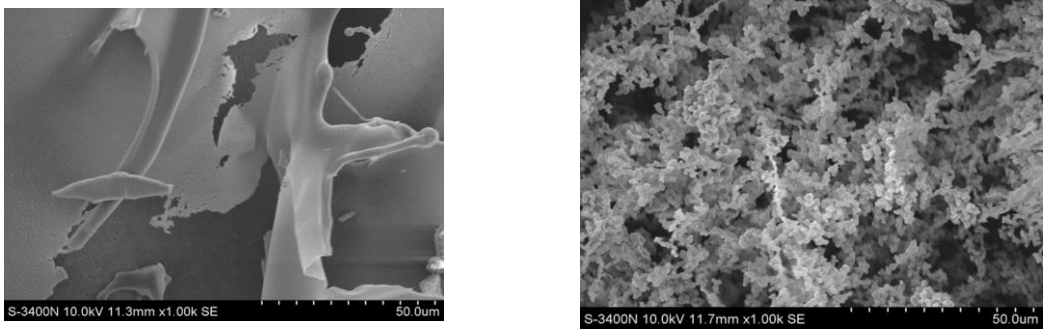



**Figure 1.** Infrared (IR) spectra of OG and FOG

*3.2. Conformational Characterization of OG and FOG*

To further dissect the impact of HPMF treatment on the morphological and molecular conformational characteristics of oat β-glucan, this study employed SEM and AFM to systematically characterize the microscopic morphologies of OG and FOG.

As illustrated in Figure 2a and 2b, prior to HPMF treatment, oat β-glucan exhibited a densely packed, lamellar architecture. Post-treatment, however, it underwent a striking transformation into a sparse, chain-bead-like structure. This morphological shift can be primarily attributed to the synergistic effects of high shear forces and cavitation phenomena generated during HPMF treatment. These forces disrupt the intermolecular hydrogen bonds and van der Waals interactions within β-glucan, effectively reconfiguring its spatial conformation. Consequently, electron microscopy images depict fragmented particle morphology and collapsed surface structures[8]. Additionally, high-pressure treatment disrupts the ordered crystalline lattice of β-glucan, reducing its crystallinity and inducing a transition of molecular chains from an ordered helical state to a more disordered[9], flexible conformation, which manifests as a looser and irregular architecture under the electron microscope.



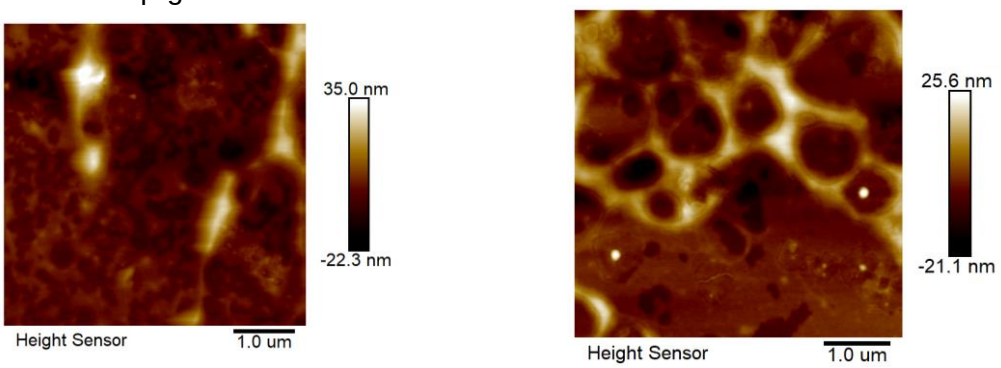

(**a**)**OG** (**b**)**FOG**

**Figure 2.** Micro - morphology diagrams of OG and FOG (a) SEM of OG; (b) SEM of FOG.

The AFM imaging data presented in Figure 3a and 3b further reveal that HPMF treatment, through mechanical shearing, effectively breaks intermolecular hydrogen bonds and physical cross-links. This process diminishes aggregate sizes and promotes the dissociation of molecules into individual entities, thereby enabling the visualization of distinct single-chain morphologies in AFM images[10]. Notably, HPMF-treated oat β-glucan displays highly flexible linear polymer chains.

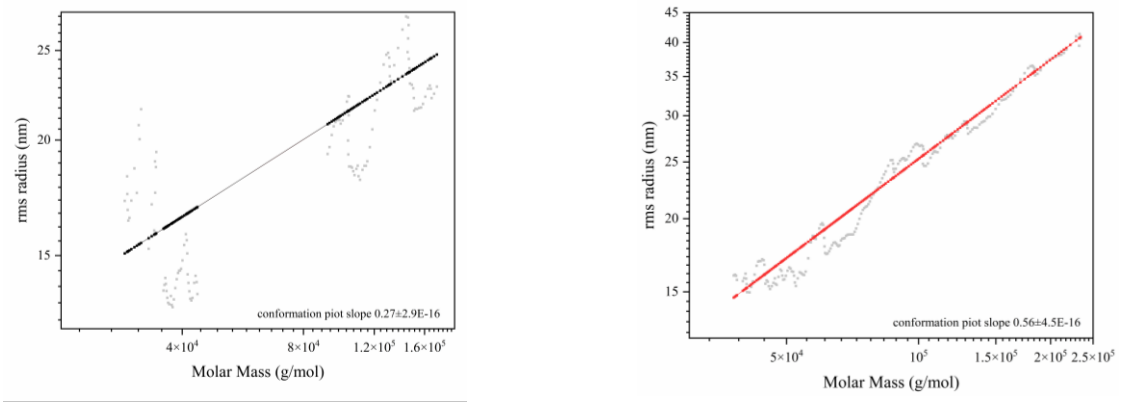
Molecular flexibility, defined as the capacity of polymer or macromolecular chains to alter their spatial conformations via single-bond internal rotation, is intrinsically governed by molecular structural parameters such as bond length, bond angle, substituent size, and polarity[10]. This property critically influences dissolution behavior, rheological properties, and biological activities. Flexible molecules typically adopt random coil conformations in solution, resulting in larger hydrodynamic volumes, whereas rigid molecules tend to assume extended or helical forms with reduced dimensions. Leveraging the Mark-Houwink principle in conjunction with gel permeation chromatography represents the gold standard for evaluating the molecular conformations of polysaccharides[11]. This approach enables

precise characterization of molecular conformation, size, and shape in solution (with log(molecular weight) as the abscissa and log(root mean square radius) as the ordinate, and the slope can be used as a basis for judging the molecular configuration: slopes of 0.33, 0.50-0.60, and 1.0 correspond to rigid spherical, flexible random coil, and rigid rod-like conformations, respectively)[13]. As depicted in Figure 4a and 4b, the molecular configuration diagrams of OG and FOG reveal slopes of 0.27 and 0.56, respectively.These values indicate that HPMF treatment significantly enhances the molecular flexibility of oat β-glucan, facilitating a transition from a rigid to a flexible state. Collectively, these findings underscore HPMF as an exceptionally efficient methodology for inducing flexible modifications in oat β-glucan.




(**a**)**OG** (**b**)**FOG**

**Figure 3.** Micro - morphology diagrams of OG and FOG (a) AFM of OG; (b) AFM of FOG.




(**a**)**OG** (**b**)**FOG**

**Figure 4.** Molecular configuration diagrams of OG and FOG (a) Molecular configuration diagram of OG; (b) Molecular configuration diagram of FOG.

*3.3. Evaluation of the Cell Repair Efficacy of OG and FOG*

Numerous previous studies have established that oat β-glucan demonstrates remarkable efficacy in promoting wound healing. In the cosmetics industry, the scratch assay has become a gold-standard method for evaluating the reparative properties of cosmetic formulations. To assess the reparative potential of OG and FOG, this study employed a keratinocyte-based scratch assay, utilizing recombinant human epidermal growth factor (hEGF) as a positive control benchmark.

As illustrated in the accompanying Figure 5, both 0.1% OG and 0.1% FOG exhibited notable reparative effects. However, FOG demonstrated superior performance, with its reparative efficacy rivaling that of hEGF. Specifically, FOG facilitated an 82% closure of the scratched area within 24 hours, significantly outperforming OG, which only achieved a 53% healing rate. This differential efficacy may be attributed to the enhanced molecular flexibility of FOG. According to recent research, flexible macromolecules exhibit heightened affinity for cell membrane receptors, enabling them to engage with multiple receptor types

simultaneously[15]. This multi-receptor activation mechanism likely underlies FOG's potent reparative capabilities, positioning it as a promising candidate for applications requiring accelerated cellular repair.

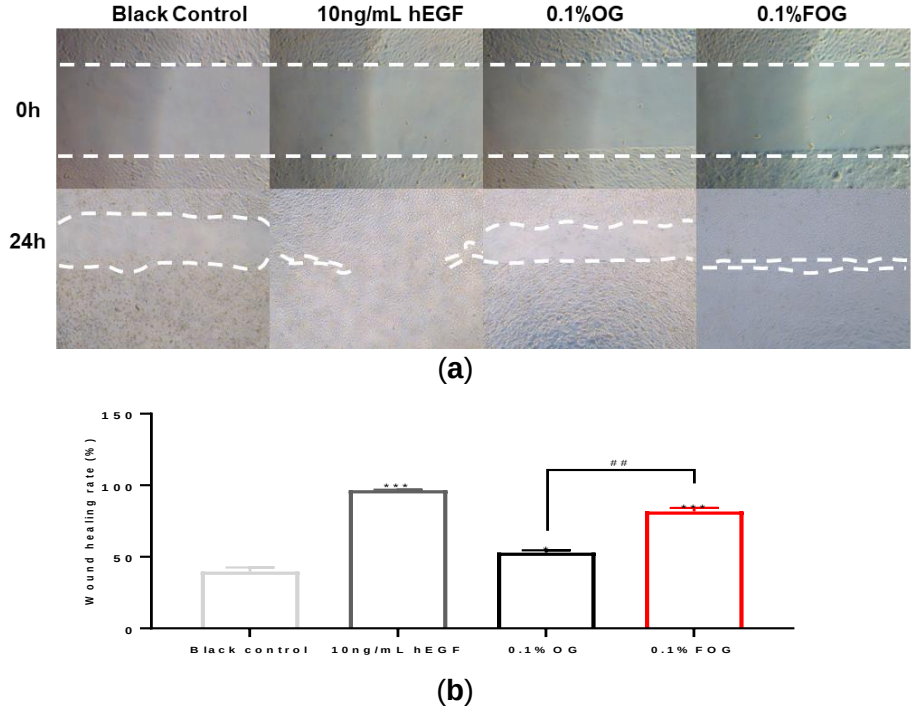



**Figure 5.** Cell scratch healing diagram (a) Microscopic images of scratch healing (100X); (b) Diagram of the relative scratch healing rate.

*3.4. Evaluation of the Anti-aging Efficacy of OG and FOG on Cells*

To investigate the effects of OG and FOG on skin anti - aging and to explore whether the flexible oat β - glucan obtained through HPMF modification exhibits superior efficacy in this regard, this study employed the ELISA method to measure the expression level of type III collagen in a natural fibroblast model treated with OG and FOG. The results are presented in Figure 1. Both OG and FOG were found to stimulate the synthesis of type III collagen in fibroblasts. Type III collagen, also known as "baby collagen", is a key determinant of skin elasticity. These findings suggest that oat β - glucan holds potential for enhancing skin elasticity. Notably, the flexible oat β - glucan FOG, which has been modified by HPMF, demonstrated a significantly stronger promotion of type III collagen synthesis compared to OG, achieving a comparable effect to that of hEGF. This indicates that HPMF can significantly enhance the anti - aging efficacy of oat β - glucan.

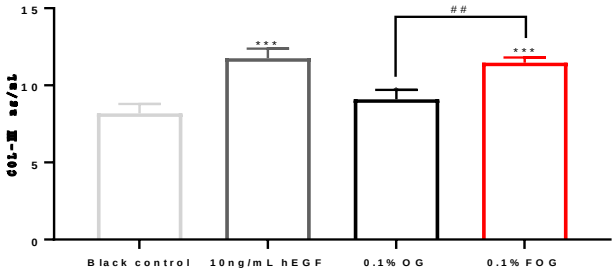



Figure 6. Diagram of the Expression Level of Type III Collagen in Fibroblasts

*3.5. Evaluation of the Repair Efficacy of OG and FOG on Human Skin after Fractional Laser Treatment*

In cell experiments, we have verified that FOG exhibits significantly superior repair and anti-aging efficacy compared to OG, primarily attributed to its unique flexible conformation. To

further explore the repair efficacy of flexible oat β-glucan on human skin, this study investigated the restorative role of FOG in human facial $CO^2$ fractional laser treatment. Additionally, its repair efficacy was compared when used in combination with hEGF and type III collagen, which are commonly used repair agents in medical aesthetics.
As shown in Figure 7, the application of 0.05% FOG can significantly accelerate scab shedding and promote skin repair, achieving a comparable effect to 5000 IU of hEGF. It can notably reduce the recovery period of ablative fractional laser treatment from 7 days to 5 days. From the enlarged local view in Figure 7, it is evident that FOG can significantly accelerate skin re-epithelialization, with an effect equivalent to that of EGF. This conclusion aligns with the results of the cell experiments presented in Figure 5. Since FOG can remarkably promote the migration of basal layer cells, it accelerates scab shedding and shortens the recovery period.

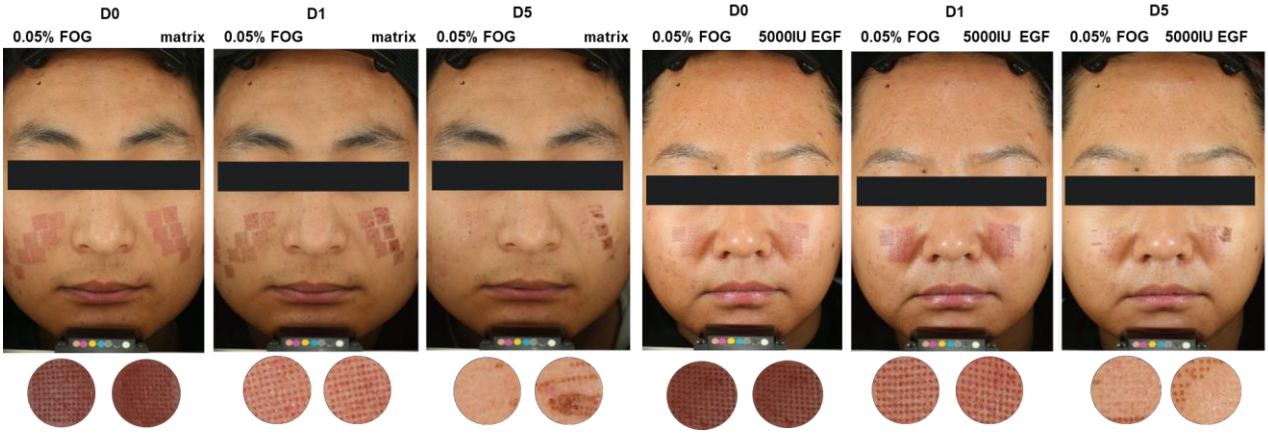



**Figure 7.** Skin VISA CR standard light images after fractional laser treatment

To further investigate the repair effect of FOG, this study utilized skin ultrasound and a skin elasticity tester to measure the epidermal thickness, dermal density, and total skin elasticity of the fractional laser-treated area. As depicted in Figure 8, FOG significantly increased skin density and thickness, accelerating the recovery of the damaged region. After 14 and 28 days of FOG application, the total skin elasticity increased by 11% and 20%, respectively, while the dermal density increased by 17% and 23%, respectively. The enhancement in total skin elasticity can be attributed to FOG's role in elevating dermal density. This finding aligns with the results presented in Figure 6; by promoting fibroblasts to secrete type III collagen, FOG enhances skin density and elasticity. These results collectively demonstrate that the application of 0.05% FOG can significantly facilitate the repair of damage caused by ablative fractional laser treatment, achieving an efficacy comparable to that of the combination of hEGF and type III collagen.

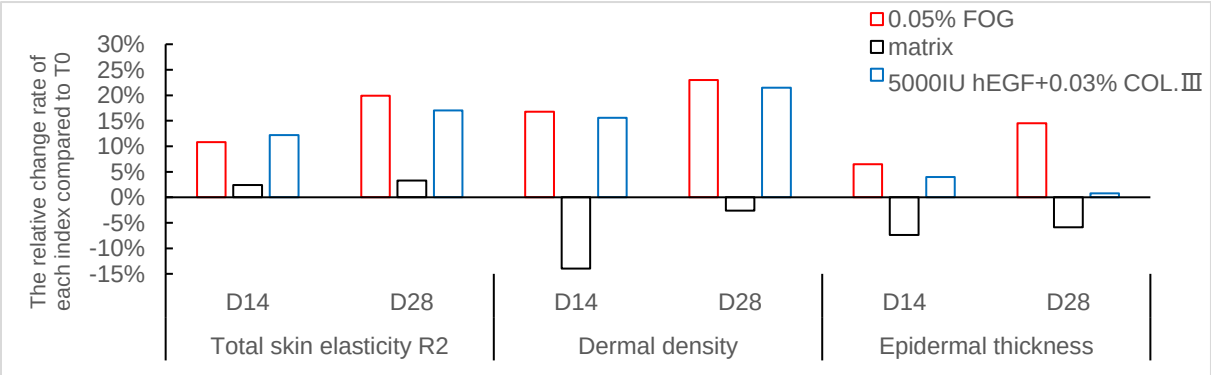



**Figure 8.**Quantitative diagrams of skin elasticity, dermal density, and epidermal thickness relative to the state at T0

*3.6. Evaluation of the Efficacy of OG and FOG in Repairing and Anti-aging for Sensitive Skin*

Given the outstanding repair capabilities and remarkable effect in enhancing skin elasticity that FOG has demonstrated in its application following fractional laser treatment, this study further aimed to validate its efficacy in skin repair and anti-aging among individuals with sensitive skin. The validation primarily focused on phenotypic indicators such as crow's feet and skin elasticity.

As illustrated in Figure 9, the application of a 0.05% FOG facial cream led to a significant reduction in both the depth and length of crow's feet. Additionally, it exhibited the beneficial effect of lifting the facial muscles.

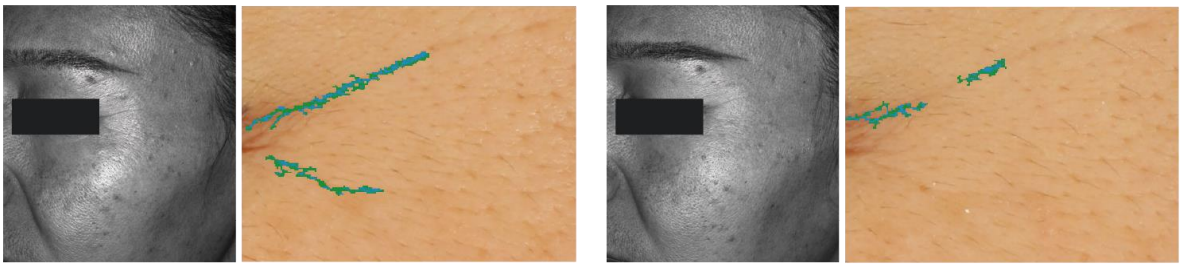


**Figure 9.**Skin EVASKIN image and the standard light image of crow's feet around the eyes taken by skin VISIA CR device

After 28 days of using the 0.05% FOG facial cream, there was a notable increase in dermal density, rising by 18%. Concurrently, the total skin elasticity index R2 also witnessed a significant improvement, with an increase of 26%.

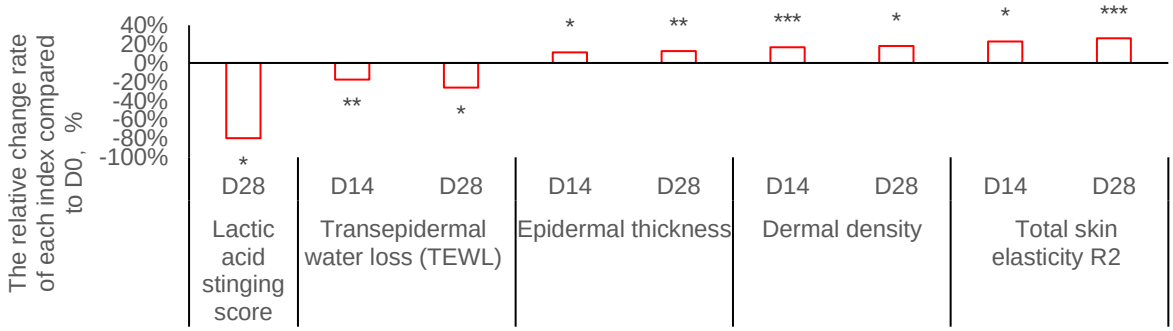



**Figure 10.**Quantitative diagram of the change rate of each skin index compared with that at D0

Given that poor skin tolerance is the most characteristic symptom of sensitive skin, we employed the lactic acid stinging score method to assess the skin tolerance of individuals with sensitive skin. As depicted in Figure 10, after 28 days of FOG application, the lactic acid stinging score significantly decreased by 80%.

These findings collectively validate that FOG's unique flexible structure endows it with exceptional repair and anti-aging properties. It effectively addresses the skin sensitivity issues faced by those with sensitive skin, offering a reliable solution for improving skin resilience and overall skin health.

## 4. Discussion

There is no doubt about the functions and advantages of oat β-glucan in the field of skin care. Over its more than 20-year application history in the cosmetics industry, it has been found to possess multiple functions such as moisturizing, repairing, soothing, and anti-aging. Regarding the "brick-wall and mortar" structure of the stratum corneum, oat β-glucan can carry out repairs in different aspects. For the "mortar" component, oat β-glucan can promote the expression of caspase3, thereby facilitating the degradation of filaggrin into natural moisturizing factors (NMF), thus replenishing the mortar[16]. For the more crucial repair of the

"brick wall" (keratinocytes), oat β-glucan is capable of promoting the migration and differentiation of keratinocytes, enabling them to form a more compact and orderly stratum corneum structure[17], and thus achieving the repair of the "brick wall".The unique linear molecular structure of oat β-glucan endows it with good transdermal properties. Li Xiaopeng's research shows that oat β-glucan extracted by three methods, namely water extraction, enzymatic extraction, and fermentation, can penetrate the skin[8]. Meanwhile, oat β-glucan can stimulate skin fibroblasts to synthesize collagen, which proves that oat β-glucan can effectively achieve anti-aging effects[18]. In terms of clinical trials, a high-efficiency wound-healing composite hydrogel prepared from Egyptian oat polysaccharides containing β-glucan has been used to repair the facial skin after fractional laser treatment in medical aesthetics.Oat β-glucan belongs to natural polysaccharides, and its molecular structure is a key factor affecting its efficacy. Due to its complex structure, the academic community has not yet fully understood the structure-activity relationship of oat β-glucan in skin care. Existing research results indicate that low-molecular-weight oat β-glucan has better anti-inflammatory and antioxidant effects, while high-molecular-weight oat β-glucan has stronger immune activation ability. Akkerman fermented oat β-glucan using endo-β-glycosidase, and the results showed that the fermented oat β-glucan with fewer β-1,4-glycosidic bonds had a stronger ability to activate the receptor Dectin-1.

In this study, it was found that even without significant changes in molecular weight, the enhancement of activity can be achieved by merely altering the spatial conformation of the polysaccharide. This is because polysaccharides, depending on their structures and substituents, exist in various forms such as spherical, rod-like, and random coil in solution. Different conformations in solution also have different biological functions. For example, the triple-helix structured β-glucans from fungi and yeasts possess stronger immune activation ability, and the flexible conformation (random coil) of hyaluronic acid has a stronger binding ability to cell membrane receptors. Chen Xiaoyu's research shows that the Poria cocos β-glucan with a more extended and flexible conformation obtained through phosphorylation modification has stronger anti-tumor activity. Similar research results have also been obtained for oat β-glucan. The highly flexible oat β-glucan obtained through sulfation modification exhibits new anticoagulant activity, and Zhang Qiyu's research also proves that the highly flexible oat β-glucan obtained through sulfation modification has stronger antioxidant function due to its enhanced binding ability to cell membrane receptors.

However, traditional flexible modification methods mainly focus on chemical methods such as sulfation and phosphorylation, which limits their application in the cosmetics industry. This study discovered that HPMF can be used to achieve the flexible modification of polysaccharides through physical means. This discovery holds certain significance for the modification of polysaccharide active substances in the cosmetics industry. However, this article fails to clarify why the HPMF process can achieve the flexible modification of oat β-glucan. Further research is also needed on the mechanisms of flexible modification by HPMF and the mechanisms underlying the enhanced efficacy of flexible oat β-glucan.

## 5. Conclusion

The results show that flexible oat β-glucan (FOG) can be prepared by treating OG with HPMF, and this treatment has no significant impact on its primary structure and main functional groups. The FOG obtained through modification exhibits superior effects in skin repair and anti-aging compared to OG, with improvements of 30% and 26% respectively. In human experiments, FOG demonstrates excellent efficacy in accelerating the repair of damages caused by medical aesthetics (comparable to the effect of hEGF) as well as in the repair and anti-aging of sensitive skin.

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