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Anti-Wrinkle Therapy: Significant New Findings in the Non-Invasive Cosmetic Treatment of Skin Wrinkles with Beta-Glucan

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Abstract

Oat beta-glucan is a water soluble, linear polymer of glucose consisting of 1,4 (70%) and 1,3 (30%) linkages with an average molecular weight of 1×10^6 Da. Scientific reports indicate beta-glucan is a film-forming moisturizer, a biological response modifier, and a promoter of wound healing. Our objective was to study the penetration of oat (1,4:1,3) beta-glucan in human skin models and to evaluate clinically its efficacy for reducing fine-lines and wrinkles.

Penetration studies performed on human abdominal skin used a single application of 0.5% beta-glucan solution at a dose of 5 mg per cm². The results showed that beta-glucan, despite its large molecular size, deeply penetrated the skin into the epidermis and dermis.

A clinical study of 27 subjects was performed to evaluate the effects of beta-glucan on facial fine-lines and wrinkles. After 8 weeks of treatment, digital image analysis of silicone replicas indicated a significant reduction of wrinkle depth and height, and overall roughness.

This work is the first *ex vivo* and *in vivo* demonstration of the physiological effects of beta-glucan in the penetration and restructuring of human tissue. The study supports the use of oat beta-glucan in the care and maintenance of healthy skin and the cosmetic treatment of the signs of aging.

Keywords: Beta-glucan, skin penetration, wrinkles, anti-aging

INTRODUCTION

Oat has a long history of safe use to provide fast, temporary relief of the itching, redness, and pain associated with many minor skin irritations such as poison ivy/oak/sumac, insect bites, and allergy [1]. In the cosmetic application of beta-glucan, consumers have described various benefits including excellent, sustained moisturization properties together with an improved, smoother appearance of the skin. In recent years new wound product applications for beta-glucan have been found in the management of partial thickness burns, shallow abrasions, and laser treatment [2, 3]. It has been reported that topical glucan administration enhances wound healing by increasing macrophage infiltration into the wound milieu, stimulating tissue granulation, collagen deposition, and re-epithelialization, together with increasing the tensile strength of the recovered wound [4, 5].

Laboratory experiments using beta-glucan from cereal (1,4; 1,3 linear glucose polymer) and fungi (1,3; 1,6 branched glucose polymer) indicated that all beta-glucans are biological response modifiers, with oat beta-glucan producing the greatest cytokine induction activity in macrophages [6, 7].

The mechanism by which glucan or glucan-induced immunomodulators enhance wound repair has remained elusive. We do know that beta-glucan receptors exist on mammalian macrophages and fibroblasts [4, 8]; the effect of glucan on wound repair is speculated to involve macrophage release of wound growth factors with the further direct and indirect modulation of fibroblast activity, including collagen biosynthesis.

In the case of wounds, the disruption of the dermal barrier gives clear and open access of macrophages and fibroblasts to topically applied beta-glucan. It remained to be determined if cellular effects could

be achieved through normal, intact skin and if the structure of aged skin could be affected positively through the cosmetic application of beta-glucan.

EXPERIMENTAL

Oat (1,4; 1,3) beta-glucan

Beta-glucan is the soluble fiber found in the cell walls of oat kernels (**Figure 1**). Structurally, oat beta-glucan is a linear polymer of glucose consisting of 1,4 (70%) and 1,3 (30%) glycosidic linkages (**Figure 2**).

The beta-glucan used in the present studies was extracted from oat and supplied as a clear, viscous, 1% solution (Symrise Inc., New Jersey). Briefly, the extraction method comprised the aqueous, mild alkali (pH 9.2) extraction of beta-glucan from oat bran, followed by the removal of protein, and ultrafiltration through a 0.1 mi-

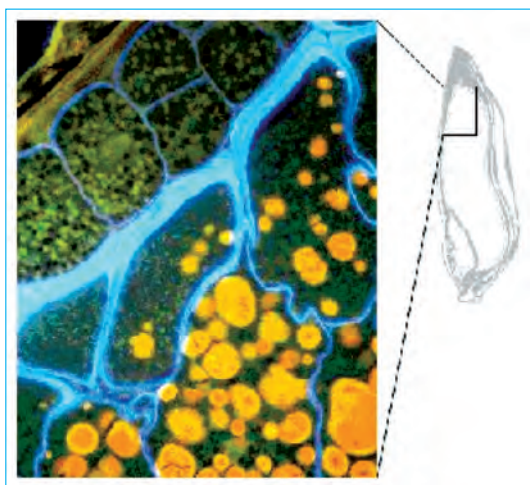


Figure 1: Fluorescent stained section of an oat kernel. The beta-glucan present in the cell wall of the oat fluoresces a brilliant blue when stained with Calcofluor White.

cron filtration system. The resulting solution was double precipitated with ethanol and resuspended to a final concentration of 1%. The ultrafiltration of the beta-glucan solution produced a clear solution confirmed by a low turbidity (<40 Nephelometric Turbidity Units: NTU). The molecular weight range of the beta-glucan was determined to be $0.5 \times 10^6 - 1.0 \times 10^6$ Da as measured by the method of Wood [9].

Skin penetration study

In the first study we examined the dermal penetration of (1,4; 1,3) beta-glucan into sections of surgically-removed, human abdominal skin. The penetration of beta-glucan was visualized using Calcofluor White, a beta-glucan specific fluorescent stain. The use of Calcofluor White also allowed semi-quantitative measurement of beta-glucan penetration with fluorescence densitometry [10, 11]. Sections of abdominal tissue were re-

moved surgically without the subcutaneous fat. The skin was sliced to fit a penetration and deposition chamber based on a Franz Diffusion Cell. The skin sections were first deep frozen by liquid nitrogen and sterilized by gamma-radiation, which destroyed all yeast and fungal elements that could interfere with the assay. After irradiation, the skin-section was thawed and the specimens were inspected for integrity before use with a pressure test.

Next, the skin section was conditioned with respect to surface temperature and moisture content. This condition was achieved by pre-heating the liquid medium in the test chamber and adjusting the air flow through the chamber's ventilation channel. A macroscopic and physical examination of the skin specimen was carried out before the test to ensure suitability, and the area of the test application site was 10 cm^2 for all samples. During testing, the skin specimen was supplied with a uniformly circulated nutrient medium, which rinsed its lower surface. The experimental conditions were non-occlusive.

The test procedure involved one application of 0.5% (w/w) beta-glucan solution using a micro dose applicator at a dose of 5 mg per cm^2 of skin. After 8 hours of incubation, the skin tissue was deep frozen. It was then cut into thin slices and air dried. Then the skin was cut from lower to higher possible concentration, meaning deeper dermis to horny layer.

The specimens were placed on thin glass slides and allowed to dry. One drop of Calcofluor White (Bactidrop™, Remel, Lenexa, KS, USA) was added and stained

for 30 seconds. The excess stain was removed by washing with deionized water. The specimens were then examined using a fluorescent microscope with an excitation wavelength ranging between 400 – 500 nm and a peak of 440 nm. Untreated skin was used as the control.

The tests were done simultaneously with two samples and one control for each volunteer skin. All tests were repeated with the skin of five volunteers.

Anti-aging study

In the second study, we performed a clinical evaluation of the capacity of beta-glucan to alleviate the extrinsic signs of aging. The study was conducted in Colorado during the winter months to provide a dry environmental challenge together with a high exposure rate to UV.

The test was conducted on a panel of 27 subjects, with two carbomer gel formulations; one contained 0.1% (w/w) (1,4; 1,3) beta-glucan and the other was placebo. The subjects applied the randomly assigned products twice daily, using a half-face design. The subjects observed a 3-day conditioning period immediately prior to baseline measurements. Each of the 27 subjects treated their left and right sides of the face, twice daily for eight weeks. After 8 weeks of treatment, the skin was evaluated for changes from baseline values of various parameters including fine lines, wrinkles and roughness.

The clinical study included subjective and objective assessments which were recorded at baseline and 2, 4, and 8 weeks. For the evaluation of fine-lines and wrinkles, silicone replicas of the outer canthus of the eye area (crow's feet) were subjected to digital image analysis by expert graders. Macrophotography was also

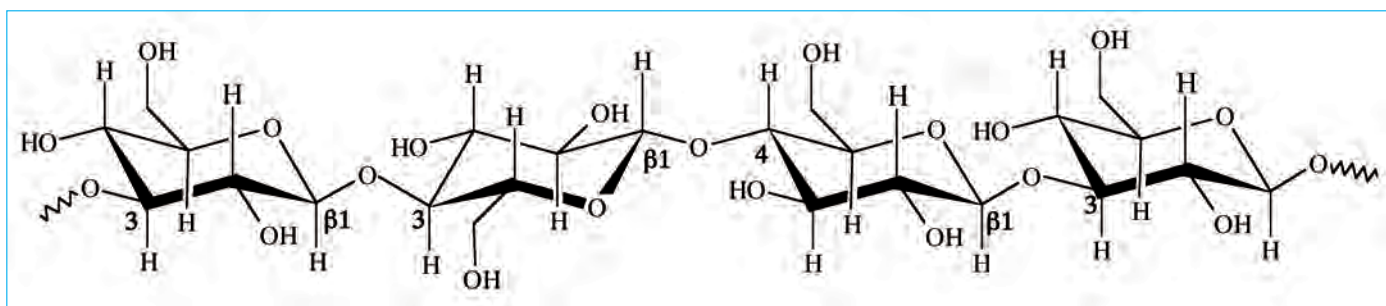


Figure 2: Chemical structure of oat beta-glucan showing the beta 1,4 and beta 1,3 glycosidically linked glucose polymer structure.

used to evaluate the changes in fine lines and wrinkles.

RESULTS AND DISCUSSION

The results of the skin penetration study showed that (1,4; 1,3) beta-glucan had penetrated the skin into the epidermis and dermis (**Figures 3 and 5**). No fluorescence staining occurred in the control skin sec-

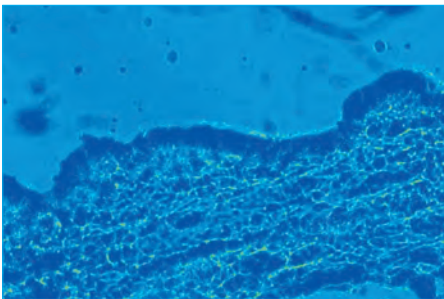


Figure 3: Photograph of dermis skin section treated with 0.05% (w/w) (1,4; 1,3) beta-glucan solution (magnification x125).

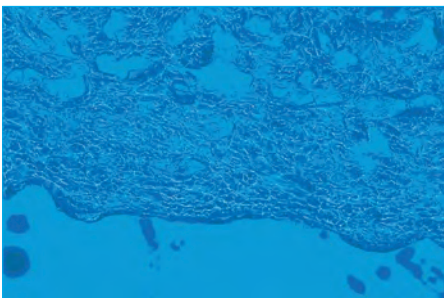


Figure 4: Photograph of the control dermis skin section (magnification x125).

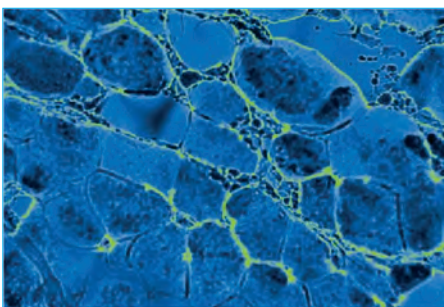


Figure 5: High magnification photograph of epidermis skin section treated with a 0.05% (w/w) solution of beta-glucan. Note that beta-glucan staining is associated with the inter-cellular matrix indicating that the beta-glucan permeates the skin by passing between cells rather than passing through cells directly (magnification x250).

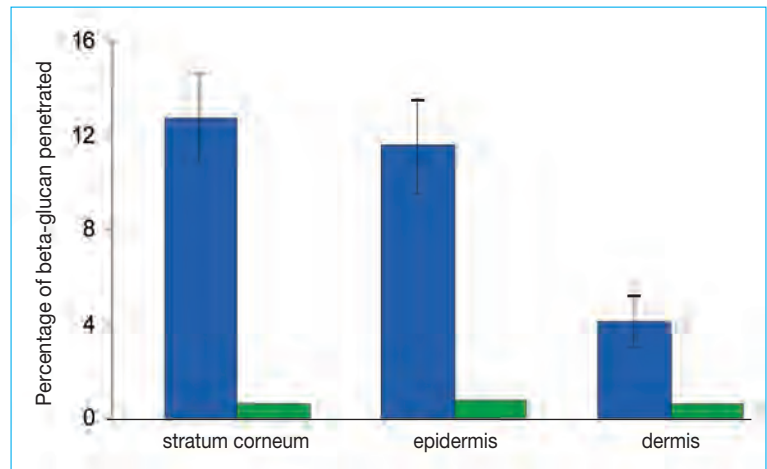


Figure 6: Graphical analysis of the fluorimetric data obtained from the skin penetration study. The blue bars represent the beta-glucan treated skin and the green bars represent the control skin. The results indicate that beta-glucan is able to penetrate into the lower levels of the skin, and therefore is able to interact with the fibroblasts and other structural elements.

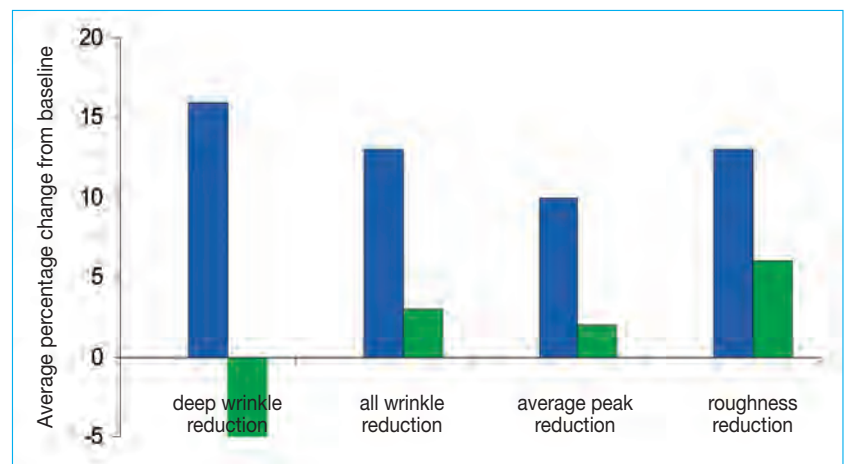


Figure 7: Graphical analysis of the facial results of the clinical trial obtained through digital picture analysis after 8 weeks. The blue bars represent the beta-glucan treated skin and the green bars represent the control skin.

tion which was not treated with beta-glucan (**Figure 4**). Quantitative assay of the fluorimetric staining indicated that a significant portion of the product (28.5% of the applied beta-glucan) had entered the skin (**Figure 6**).

The clinical trial results indicated a higher incidence of improvement with (1,4; 1,3) beta-glucan than with the placebo. **Figure 7** shows the average percentage change of the selected parameters from the baseline, compared to the treatment with placebo. The silicone replicas after the test period demonstrated a smoothing of the cutaneous surface after 8 weeks of treatment with (1,4; 1,3) beta-glucan.

Macrophotography of the left and right sides of the face also showed a reduction in lines and wrinkles.

These results represent remarkable new findings which will contribute to our understanding of the interaction of skin with beta-glucan, and the ability of beta-glucan to penetrate the skin deeply and elicit cellular changes.

In the past, the potential ability of beta-glucan to penetrate the skin was disregarded because it was thought that the high molecular weight ($>0.5 \times 10^6$ Da) of the compound would prevent it from penetrating into the epidermis and dermis, and it would therefore be unable to inter-

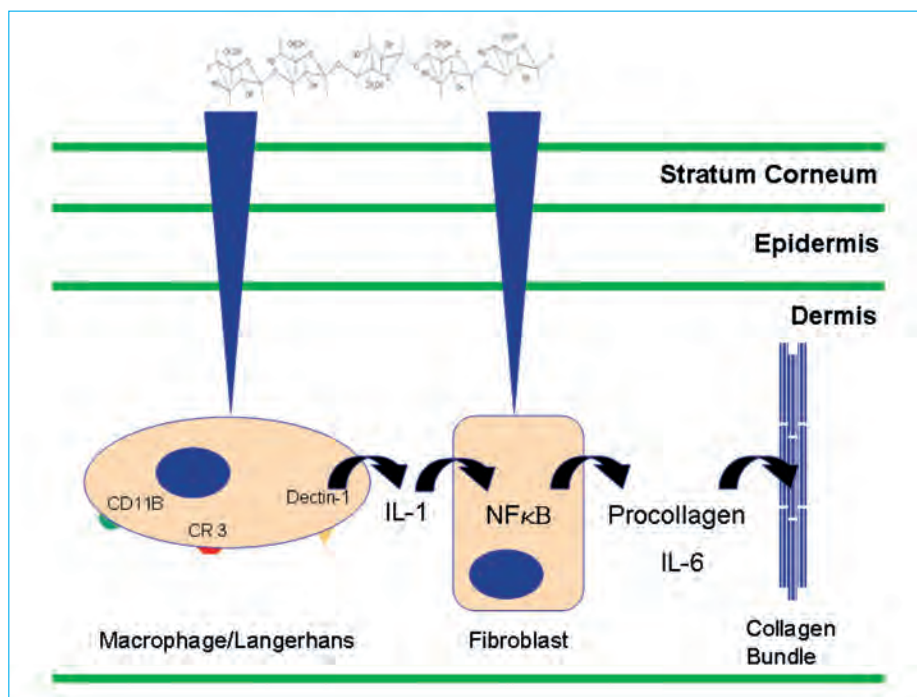


Figure 8: Proposed mechanism for the skin penetration of beta-glucan. Above the stratum corneum (horny layer) and epidermis, beta-glucan forms a thin film to promote moisturization. Within the dermis, beta-glucan is able to produce collagen synthesis through direct interaction with fibroblasts and through indirect, cytokine mediated, interaction with macrophages. Collagen synthesis is one possible mechanism by which the elasticity of the skin is enhanced.

act with macrophages and fibroblasts. Beta-glucan is able to adopt a number of conformations and is typically extracted in the form of aggregate particles > 1 μm which are clearly visible under a light microscope. It is understandable that such large particles may not be expected to enter the skin and the effects of beta-glucan were thought to be limited to the skin's surface.

However, the beta-glucan used in the present penetration study was subject to sub-micron filtration to produce an aggregate-free, low-turbidity solution with no particles visible under the light microscope. Examination of the micrographs in **Figure 5** shows that the beta-glucan used in our study does not enter the skin by direct passage through the cells of the epidermis and dermis, but instead works its way into the skin by passing through the inter-cellular matrix. Such a process may be facilitated by a diffusion gradient and by lipid and phospholipid interactions. Interactions of beta-glucan with lipids are known and are the basis of the health-enhancing, lipid-controlling properties recognized by the FDA [12].

Having penetrated the skin to the dermis, beta-glucan is able to interact with specific cells, namely macrophages and fibroblasts. Results of *in vitro* experiments have demonstrated that beta-glucan interacts with macrophages to induce the production of IL-1, which indirectly promotes the production of procollagen by fibroblasts. In addition, beta-glucan has been shown to interact with fibroblast receptors, which results directly in the production of procollagen [13, 14]. The conversion of procollagen to collagen and its incorporation into collagen bundles would result in the type of effects noted in our clinical study, specifically the facial skin tightening leading to a reduction of fine lines and wrinkles.

Questionnaires and subject follow-ups indicated that the effects on fine lines and wrinkles associated with use of beta-glucan treatment were long-lived but not permanent. With normal cellular turnover, there was an appearance of fine lines. It may be speculated that the continued use of products containing beta-glucan would result in a sustained improvement of appearance.

The results presented in the present study offer a cosmetic alternative to other more invasive treatments aimed at the reduction of fine lines and wrinkles in an aging population. Injectable fillers like collagen – either from human, bovine, or porcine sources – are common, and recently hyaluronic acid fillers have also been introduced. Such fillers produce temporary, soft tissue with effects that last on average for 3 to 4 months. With a similar duration of effect, the cosmetic use of *Botulinum* toxin type A has been reported to have increased multifold since 1997 [15]. Actives like retinoic acid and coenzyme Q10 are also used for the treatment of wrinkles [16-18]. The regular and frequent use of cosmetics containing oat (1,4; 1,3) beta-glucan is a new and exciting tool in the fight against the signs of aging.

CONCLUSION

Oat (1,4; 1,3) beta-glucan is a natural active ingredient offering significant performance-enhancing properties for personal care applications. Our studies have shown that the molecule, despite its considerable molecular weight, is able to enter the stratum corneum and epidermis and penetrate deep into the dermis. The observed effects of beta-glucan on tissue restructuring and wrinkle reduction are most likely effects mediated by fibroblast stimulation and collagen deposition in the dermis. These unique properties make oat beta-glucan a promising and effective ingredient for cosmetics.

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