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Potential of Skin GAG-Inducing Active Ingredient for Scalp Care: Enhancing Hair Follicle Health from the Bulb

Tim TSAO [1], Sebastien THIBAUT [2], Ray MA [3], Thomas BORNSCHLOEGL [2], Adrien KAESER [1], Nathalie VARELA MONTEIRO [4], Tom SU [4], Hiroshi OTA [5], and Florence POURADIER [6,*]

[1] Scalp Application Domain, L'Oréal R&I, Kanagawa, Japan; [2] Discovery Domain, L'Oréal R&I, Aulnay-sous-Bois, France; [3] Evaluation Intelligence, L'Oréal R&I, Shanghai, China; [4] Scalp Application Domain, L'Oréal R&I, Saint-Ouen-sur-Seine, France; [5] Evaluation Intelligence, L'Oréal R&I, Kanagawa, Japan; [6] Evaluation Intelligence, L'Oréal R&I, Clichy, France

1. Introduction

The aging process induces significant changes in the hair follicle (HF) biology, resulting in miniaturization of terminal hair fibers. This miniaturization leads to a reduction of hair mass and density, a primary concern for many consumers, particularly in aging populations within Europe, the U.S., and Japan.[1–3] Addressing age-related hair thinning and promoting the appearance of fuller hair represents an area of continued interest in hair care research.

Glycosaminoglycans (GAGs), integral components of the extracellular matrix surrounding HFs, are known to support follicular growth and function.[4,5] It has been previously reported that *C*-xylosides increase GAG synthesis.[6] Clinical observations in mucopolysaccharidosis patients, who exhibit thicker, denser hair due to elevated levels of sulfated GAGs, suggest an important link between GAGs and hair shaft properties.[7] The accumulation of heparan sulfate appears to play a more significant role in hair morphology changes than the accumulation of other GAGs, such as dermatan sulfate and keratan sulfate.[7] Based on this, modulation of specific GAG synthesis, such as heparan sulfate, in the scalp appears to be a promising area to investigate for developing treatments to combat hair thinning and promote fuller hair.

Hydroxypropyl tetrahydropyrantriol (HPTP), a novel *C*-glycosylated derivative and a well-established anti-aging active known for its ability to stimulate GAG synthesis in skin and improve the expression of several proteoglycans, including those involved in hair growth, such as syndecan-1, syndecan-4, CD44 and perlecan,[6] presents itself as a strong candidate for promoting HF health and improving hair thickness. However, further research is needed to

evaluate the effects of HPTP on the biophysical properties of the follicle, as well as the molecular and cellular mechanisms underlying its observed hair-thickening benefits.

This study elucidates the effects of HPTP on HF and fiber biophysics, and its capacity to promote hair thickness and anchoring both *ex vivo* and *in vivo*. Nanomechanical properties and keratin organization of HPTP-treated HFs were assessed *via* atomic force microscopy (AFM) and optical profilometry. The influence of HPTP on keratinization onset and its implications for overall hair thickness and quality were also investigated. Formulations for enhanced scalp penetration were developed and characterized to optimize HPTP delivery. The *in vivo* efficacy of these formulations was subsequently evaluated in a clinical study to demonstrate the hair benefits of HPTP. This comprehensive approach provides mechanistic insights into HPTP's impact on hair fiber health and substantiates its application in scalp care.

2. Materials and Methods

2.1 *Ex vivo* experiments

2.1.1 Microdissection of Hair Follicles

Individual human terminal scalp hair follicles were obtained from healthy volunteers undergoing facelift surgery, following informed consent. The surgical tissue sample was sectioned into strips approximately 5 × 10 mm. The hypodermis was carefully separated from the dermis using a scalpel. Hair follicles were then isolated from the subcutaneous fat under a stereo-dissecting microscope using fine forceps. Isolated follicles were cultured for 10 days at 37 °C in a humidified 5% CO_2 / 95% air atmosphere in 24-well cell culture cluster. The culture medium used was William's E Medium supplemented with 2 mM *L*-glutamine, 10 mg/ml insulin, 40 ng/ml hydrocortisone, and 1% antibiotic solution.

2.1.2 GAGs Analysis

Hair follicles (5 hairs per well) were incubated for 72 hours in absence (control) or in presence of the test compound. The radioactive marker [^{35}S]-sulfate was added during the last 48 hours of incubation. All experiments were carried out in $n = 3$. Following incubation, culture supernatants were collected and stored at −80 °C. The hair shafts were transferred to microtubes (5 hairs per tube) and also stored at −80 °C.

After thawing, GAGs were extracted using a chaotropic buffer. GAGs were then purified by ion exchange chromatography adsorption of anionic molecules on Q Sepharose beads and desorption of low- and medium-anionic molecules with a suitable solution.

2.1.3 AFM Analysis

The treated follicles were subjected to a concentration of 1 mmol/L of HPTP for 6 days, with regular photographic examination under a binocular microscope to measure growth, diameter, and viability. At the end of this phase, the follicles were frozen in Tissue-Tek resin

and stored at −80 °C until further use. For this study, a maximum of serial sections from 5 control follicles and 5 HPTP-treated follicles were characterized, for a total of 26 sections.

On the day of analysis, the follicles were cut into 10 µm thick longitudinal sections using a Leica CM3050 Cryotome. The sections were placed on a microscope slide and immediately placed in PBS (Gibco). The sample was tested in immersion with an AFM “Icon-Dimension” (Bruker) using the “ScanAsistFluid” levers with a tip radius of 20–60 nm and a stiffness K_c = 1.1 N/m. Different 10 × 10 µm zones were selected in the axis of the bulb, avoiding the medulla, spaced 100 µm apart. 256 curves were made in each zone, allowing for mechanical mapping. Each curve is analyzed with the theoretical Hertz model (the discussion of the different models is the subject of a separate report), using the SPIP software, which yielded 256 Young's modulus values per 10 μm^2 area. The values obtained were grouped in the form of a box plot presenting the measurements in logarithmic coordinates.

2.2 Formulation

2.2.1 Sample Preparation

The formulation comprised an aqueous phase (water, HPTP, pentylene glycol, propanediol, glycerin, hydroxypropyl guar, polysorbate-21), an oil phase (oleyl alcohol, isopropyl myristate, menthol, fragrance), and ethanol. The aqueous phase was prepared by sequentially adding and dissolving each water-soluble component into water. Subsequently, after the aqueous phase became completely solubilized, oil-phase components were individually introduced and incorporated under continuous stirring. Finally, ethanol was added, completing the formulation process. All additions were performed at room temperature.

2.2.2 Stability of the formula

Stability of the formulated hydroalcoholic lotion was assessed at 4°C, 25°C, 37°C, and 45°C over two months. Evaluations were conducted at initial preparation (T_0), one week, one month, and two months for visual appearance (clarity, phase separation), viscosity, and pH value. Viscosity measurements were performed using a RHEOMAT R180 viscometer, while pH value was determined using a calibrated pH meter. Pre-defined acceptance criteria for each parameter were established based on typical product specifications.

2.3 Clinical Test Methods

2.3.1 Subjects

Female subjects aged 20–50 years with self-perceived thin hair were recruited for a clinical study evaluating the efficacy of a cosmetic active combination. Inclusion criteria included a measured hair diameter of ≤82 µm, self-reported scalp aging concerns (*e.g.*, hair fall), and a vellus hair proportion exceeding 10%. The 82 µm threshold was established based on a

previous scalp typology study of 400 Asian females, where the average hair diameter of those self-reporting "very thin" to "thin" hair was 82 μm. Furthermore, literature suggests a healthy terminal:vellus hair ratio of 25.3:1 in Asian populations;[8,9] thus, a doubled vellus proportion was considered indicative of hair thinning.

Subjects were required to have a daily hair washing habit to maintain consistent product application. They provided informed consent for hair shaving and refrained from other hair treatments three months before and during the study. Exclusion criteria included pregnancy, severe scalp disorders, and relevant adverse medical history. The study protocol adhered to the principles of the Declaration of Helsinki and received ethical approval from the Ethical Committee at Shanghai China-norm Quality Technical Service Co., Ltd.

2.3.2 Study Design

Following hair diameter pre-screening, enrolled subjects (n = 40) were assigned to use the topical HPTP serum daily to a semi-dry scalp after washing with a standard shampoo, incorporating gentle massage. Treatment duration was six months, with clinical measurements taken at 1.5 months (T_{6wk}), 3 months (T_{12wk}), and 6 months (T_{24wk}).

2.3.3 Efficacy Evaluation

Efficacy was assessed through a combination of clinical and instrumental measurements related to hair fall, hair growth, and scalp health. Hair fall was evaluated by experienced dermatologists using a standardized combing and pulling test. Hair growth was assessed *via* phototrichogram to determine hair density and telogen hair percentage. Visual hair thickness was evaluated using image analysis of scalp hair fibers captured with a specialized scalp probe. Scalp health parameters, including scalp softness and transepidermal water loss (TEWL), were also measured. Scalp softness, an indicator of the scalp elasticity, was measured using the identometer IDM 800 (Courage+Khazaka Electronics, German). Subjective assessments of product efficacy were collected through online questionnaires throughout the study.

2.3.4 Statistical Analysis

Statistical analyses were performed using SPSS Statistics (SPSS Inc., Chicago, Ill., USA). All tests were two-sided, and statistical significance was set at $\alpha = 0.05$. Descriptive statistics for each treatment group included the number of observed values, mean, standard deviation, median, interquartile range (IQR; Q1–Q3), and minimum/maximum for continuous variables. Categorical variables were summarized as frequencies and percentages.

Within-group comparisons of clinical data across time points (T_0 *vs.* T_{6wk}, T_{12wk}, T_{24wk}) were conducted using the Wilcoxon signed-rank test. For instrumental data, normality of data was checked by visualization of the data distribution. Normally distributed data were analyzed using repeated-measures ANOVA, followed by Dunnett's post-hoc test for pairwise

comparisons against baseline (T_0). Non-normally distributed instrumental data were analyzed using the Wilcoxon signed-rank test.

Between-group comparisons at each time point were performed using the independent samples *t*-test for normally distributed data and the Mann–Whitney *U* test for non-normally distributed data. Normality was assessed as described above.

3. Results and Discussions

3.1 Discussion on *Ex vivo* tests

3.1.1 GAGs Analysis

The effect of HPTP on sulfated GAG synthesis in HFs was assessed using radioisotope incorporation. As detailed in Table 1, treatment with HPTP led to a significant increase in the synthesis of sulfated GAGs in a dose-dependent manner. Compared to the control group, HPTP at concentrations of 10 µM, 100 µM, and 1000 µM resulted in increases of 11%, 100%, and 590% respectively, as measured by [^{35}S]-sulfate incorporation. This data clearly demonstrates that HPTP stimulates sulfated GAG production in the *ex vivo* HF model. This upregulation is visualized in Figure 1, which presents the dose-dependent increase in radioactive sulfate incorporation.

Table 1. HPTP Effect on the synthesis of sulfated GAGs

Treatments		**Radioactive Sulfate in HFs**				
Compound	**Concentration**	[^{35}S]-sulfate (cpm)	Averaged (cpm)	esm (cpm)	% *vs.* Control	esm (%)
Contol	-	5299 6156 4563	5339	460	**100**	9
HPTP	10 µM	6341 5208 6268	5939	336	**111**	7
	100 µM	8597 10790 12632	10673	1166	**200**	22
	1000 µM	28633 17829 48060	31507	8844	**590**	166

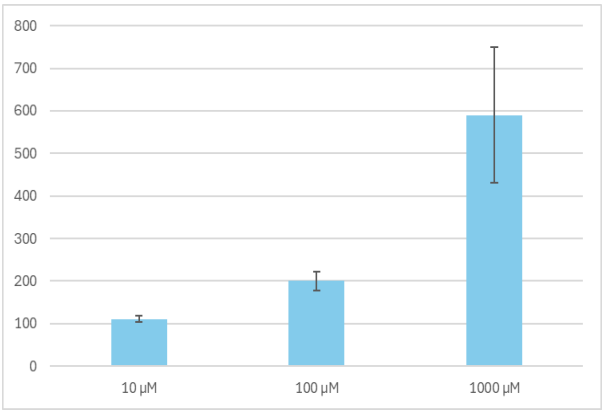
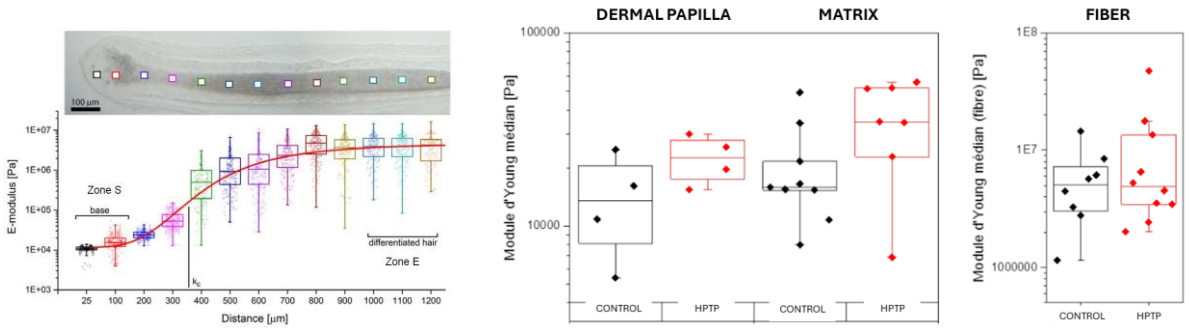


Figure 1. HPTP Effect on the synthesis of sulfated GAGs in the *ex vivo* model

3.1.2 AFM Analysis

AFM was employed to investigate the impact of HPTP on the biomechanical properties of HFs. Figure 2a presents a representative keratinization curve, illustrating the changes in Young's modulus along the length of a HF. Each data point represents the average of 256 measurements taken within a 10 × 10 µm area, and the red curve represents a sigmoidal fit to the data. As shown in Figure 2b, HPTP treatment resulted in a significant increase in the stiffness of both the dermal papilla and the surrounding matrix region, while the stiffness of the differentiated hair fiber remained unchanged. This localized increase in stiffness suggests that HPTP specifically affects the mechanical properties of the HF bulb.



(**a**) (**b**)

Figure 2. (a) Evolution of Young's modulus as a function of the distance from the base of the bulb. Top: approximate position of the 13 analysis zones on the section in optical microscopy, and bottom: typical keratinization curve of a J6 HF section. Each box groups 256 force-volume mode measurements carried out on a region of 10 µm × 10 µm. The red curve is the result of fitting the experimental data to a Hill sigmoid in log coordinates. (b) Boxplots summarizing the mean Young's modulus measurements [Pa] for control (black) or HPTP-treated (red) sections, at the level of the dermal papilla, matrix and differentiated fiber

3.2 Formulation and Delivery Optimization

The hydroalcoholic lotion demonstrated robust stability over two months under all tested temperature conditions. Visual appearance, viscosity, and pH remained within pre-defined acceptance ranges, indicating formulation stability under typical storage and handling conditions. The absence of phase separation or significant viscosity changes suggests effective ingredient compatibility within the hydroalcoholic matrix. Consistent pH further corroborates formulation stability. Therefore, the lotion is considered stable under the tested conditions.

The strategic combination of ingredients plays a vital role in optimizing the delivery of HPTP. HPTP, being a sugar-derived molecule, has high water solubility, which can hinder its penetration through the lipophilic stratum corneum. The formulation addresses this challenge through a "poor solvent" approach. The inclusion of oleyl alcohol and isopropyl myristate, both polar oils, creates a lipophilic microenvironment within the lotion, reducing HPTP's solubility in the aqueous phase and promoting its partitioning towards the lipid-rich stratum corneum.[10] This facilitates penetration into the scalp and towards the HFs.

This effect is further augmented by the inclusion of polyols like pentylene glycol, propanediol, and glycerin. By acting as co-solvents, polyols improve the solubility of the oils in the aqueous phase while also acting as penetration enhancers.[11,12] They disrupt the ordered structure of the stratum corneum lipids, creating transient pathways for increased permeation of active molecules. Ethanol serves a dual purpose as both a penetration enhancer[13] and a co-solvent, further promoting HPTP diffusion. This sophisticated delivery system is crucial for maximizing HPTP's bioavailability and efficacy.

3.3 Clinical Test

3.3.1 Study Population

The test group included a total of 48 female subjects, aged 20–50 years, with self-perceived thin hair (diameter ≤82 μm), scalp aging concerns, and a vellus hair proportion exceeding 10%, in the 24-week study. Baseline characteristics are presented in Table 2.

Table 2. Baseline characteristics of study participants

Parameter	Test Group (*n* = 48)
Age	42.6 ± 7.0
Hair density (hairs/cm^2)	151.31 ± 32.82
Mean hair diameter (μm)	81.28 ± 8.22
Vellus hair (%)	16.37 ± 5.33
Combing hair fall (hairs)	21.42 ± 5.86

3.3.2 Clinical Efficacy and Mechanistic Insights

Table 3 summarizes the changes in clinical measurements over the 24-week treatment period. A statistically significant reduction in hair fall was observed, with a mean decrease of 13.4 hairs ($p < 0.001$) in combing hair fall at 24 weeks compared to baseline. Hair thickness significantly increased by 3.4 µm ($p < 0.001$). The percentage of telogen hairs decreased from 22.9% to 19.3% ($p < 0.001$). However, the change in hair density was not statistically significant. Scalp softness improved, with a +0.31 mm increase in press depth ($p < 0.001$), and TEWL decreased by 1.2 g/h/m^2 ($p < 0.001$), indicating improved barrier function.

Table 3. Changes in clinical measurements over time

Parameter	T_0	T_{6wk}	T_{12wk}	T_{24wk}	*P* value
Combing hair fall (hairs)	21.42 ± 5.86	16.60 ± 5.17	12.85 ± 3.79	8.06 ± 3.07	<0.001
Hair density (hairs/cm^2)	151.31 ± 32.82	-	-	154.58 ± 32.88	>0.1
Mean hair diameter (µm)	81.28 ± 8.22	83.34 ± 6.75	83.43 ± 8.39	84.21 ± 8.27	<0.001
Telogen hair (%)	22.87 ± 5.29	-	-	19.28 ± 3.66	<0.001
Scalp softness (mm)	0.90 ± 0.08	1.08 ± 0.09	1.20 ± 0.05	1.22 ± 0.06	<0.001
TEWL (g/h/m^2)	14.31 ± 1.37	13.72 ± 1.33	13.09 ± 1.30	13.08 ± 1.58	<0.001

The clinical study provides compelling evidence for the efficacy of the HPTP lotion in addressing age-related hair thinning. The significant reduction in hair fall, measured by the standardized combing test, is a key indicator of improved hair anchoring and follicle health. The observed increase in hair thickness likely results from the enhanced keratinization process and improved follicle biophysics, as suggested by the *ex vivo* data.

To further understand hair thickness changes, vellus hairs[3] were analyzed using high-resolution dermoscopic images and a custom algorithm. This analysis yielded the mean hair thickness and the percentage of vellus (<40 µm) and terminal (≥40 µm) hairs, a metric we termed "thickness variability." Thickness variability improved over 24 weeks, with vellus hair percentage decreasing from 29% to 26.6%. This shift was further elucidated by examining sub-categories within the thickness variability metric: thin (40–60 µm), moderate (60–80 µm), and thick (>80 µm) hairs. This granular analysis revealed a dynamic progression towards thicker hair categories over time, indicating a holistic improvement in hair regrowth. While the change in hair density was not statistically significant in this study, it is important to note that hair density is a complex parameter influenced by multiple factors. Further studies with a larger sample size and longer duration might reveal more subtle changes in hair density.

The improvement in scalp softness, measured by increased press depth using the identometer, suggests a positive impact on scalp elasticity. Research showed the difference between vigorous (age 20s) and ageing scalp (age 50s) was huge regarding scalp softness from

1.10 mm to 0.96 mm.[14] Age-related decline in scalp softness is often associated with reduced synthetic capacity of dermal fibroblasts and subsequent collagen loss. The observed decrease in TEWL further supports the improvement in scalp health, indicating enhanced barrier function. These positive changes in the scalp microenvironment may contribute to improved hair growth and overall appearance.

The observed increase in hair thickness variability, particularly the shift towards thicker hair categories, further supports the notion of improved hair growth and follicle health. This detailed analysis provides valuable insights into the dynamics of hair regrowth and highlights the potential of HPTP to promote the development of thicker hair fibers.

4. Conclusions

GAGs are long polysaccharide chains, known to bind and modulate a large number of biomolecules involved in cellular differentiation or proliferation. These include FGF, VEGF, sonic hedgehog, BMPs, Wnt and HGF. All of these morphogenetic and growth factors were well known to be involved in HF cycling and morphogenesis. This network, by forming a specific microenvironment in the HF, could be considered as a “reservoir” for growth promoters and/or modulators able to improve HF homeostasis and growth. In this study, HPTP treatments dramatically increased the production of sulfated GAGs in the HFs, so, a such effect should have an impact on these key mediators highly involved in the health of the hair.

When we focused on the functional impact of this increase of GAGs, the first indicator that we observed is a modulation of the mechanical properties of the base of the bulb. An hypertrophy and stiffening of the matrix zone was observed by AFM, suggesting that HPTP treatment was modulating the cytoskeleton of keratinocytes. It seems that treated hair have a larger and a denser bulb than the control condition.

The development of this stable and effective HPTP lotion represents a significant advancement in scalp care. The strategic formulation, utilizing a poor solvent system and chemical penetration enhancers, optimizes the delivery of HPTP to the HFs. This approach effectively overcomes the limitations often associated with delivering hydrophilic actives to the scalp.

This research underscores the critical importance of considering both solubility and penetration enhancement strategies when designing topical formulations, particularly for scalp care. The clinical study demonstrated the efficacy of the HPTP lotion in improving multiple parameters associated with age-related hair thinning, including hair fall, thickness, and scalp health. The positive changes suggest a multi-faceted benefit of HPTP, potentially promoting a more favorable scalp environment for hair growth and improving overall hair appearance.

While this study provides strong evidence for the efficacy of HPTP in scalp care, further research is warranted. Investigating the molecular mechanisms underlying HPTP’s effects on

HF biology, including its impact on gene expression and protein synthesis, would be valuable. Long-term studies are also needed to assess the sustained benefits of HPTP use. Furthermore, exploring the synergistic effects of HPTP with other active ingredients could lead to even more comprehensive solutions for age-related hair concerns. Finally, correlating the clinical observations with histological analyses of scalp biopsies would provide further insights into the structural changes induced by HPTP treatment.

5. References

(1) Trotter, M.; Dawson, H. L. *Am. J. Phys. Anthropol.* **1934**, *18* (3), 443–456.

(2) Trotter, M. *Am. J. Phys. Anthropol.* **1930**, *14* (3), 433–445.

(3) Ishino, A.; Takahashi, T.; Suzuki, J.; Nakazawa, Y.; Iwabuchi, T.; Tajima, M. *Br. J. Dermatol.* **2014**, *171* (5), 1052–1059.

(4) Malgouries, S.; Thibaut, S.; Bernard, B. A. *Br. J. Dermatol.* **2008**, *158* (2), 234–342.

(5) He, T.; Fisher, G. J.; Kim, A. J.; Quan, T. *PLoS One* **2023**, *18* (12), e0292791.

(6) Pineau, N.; Bernerd, F.; Cavezza, A.; Dalko-Csiba, M.; Breton, L. *Eur. J. Dermatol.* **2008**, *18* (1), 36–40.

(7) Malinowska, M.; Jakóbkiewicz-Banecka, J.; Kloska, A.; Tylki-Szymańska, A.; Czartoryska, B.; Piotrowska, E.; Węgrzyn, A.; Węgrzyn, G. *Eur. J. Pediatr.* **2008**, *167* (2), 203–209.

(8) Loussouarn, G.; Lozano, I.; Panhard, S.; Collaudin, C.; Rawadi, C. El; Genain, G. *Eur. J. Dermatology* **2016**, *26* (2), 144–154.

(9) Ko, J.-H.; Huang, Y.-H.; Kuo, T. *Dermatologic Surg.* **2012**, *38* (9).

(10) Kanikkannan, N.; Singh, M. *Int. J. Pharm.* **2002**, *248* (1), 219–228.

(11) Panchagnula, R.; Salve, P. S.; Thomas, N. S.; Jain, A. K.; Ramarao, P. *Int. J. Pharm.* **2001**, *219* (1), 95–105.

(12) Lane, M. E. *Int. J. Pharm.* **2013**, *447* (1), 12–21.

(13) Gupta, R.; Badhe, Y.; Rai, B.; Mitragotri, S. *RSC Adv.* **2020**, *10* (21), 12234–12248.

(14) Lee, Y. I.; Kim, J.; Park, S. R.; Ham, S.; Lee, H. J.; Park, C. R.; Kim, H. N.; Kang, B. H.; Jung, I.; Suk, J. M.; Lee, J. H. *Ski. Res. Technol.* **2023**, *29* (8), e13433.