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ORIGINAL ARTICLE

The formulation and in vitro evaluation of WS Biotin, a novel encapsulated form of D-Biotin with improved water solubility for hair and skin treatment applications

Shaher Duchi[1] | **Paloma Rebollo Torregrosa**[2] | **Akram Hajuj**[1] | **Danit Molho**[1] | **Rawya Shkoor**[1] | **Nadeen Abo Saada**[1] | **David González Fernández**[2] | **Danny Goldstein**[1] | **Alejandro Pérez-Fernández**[2]

[1]Tagra Biotechnologies, Northern Galilee, Israel

[2]Dermaclaim Lab S.L., Valencia, Spain

Correspondence
Danny Goldstein, Tagra Biotechnologies, Northern Galilee, Israel.
Email: dannyg@tagra.com

Alejandro Pérez-Fernández, Dermaclaim Lab S.L., Valencia, Spain.
Email: alejandro.perez@dermaclaim.com

Abstract

Objective: To develop and evaluate the efficacy of WS Biotin, a novel water-soluble form of D-Biotin, for cosmetic use.

Methods: A new encapsulated form of D-Biotin was developed with the purpose of improving the water solubility of biotin. This novel form of encapsulated biotin was characterized by its physicochemical properties: particle size, D-Biotin content and solubility in water. Also, proliferation and gene expression in vitro tests in cell culture were performed to evaluate its effectiveness in promoting hair growth, an ELISA test was conducted for hair keratinization and skin lightening property was tested by analysing the intracellular melanin content.

Results: The developed WS Biotin microcapsules exhibit a particle size range of 2–30 μm with D-Biotin content of ~50% (w/w). The water solubility of WS Biotin was found to be 20-fold greater than free biotin. The obtained in vitro results indicated that WS Biotin enhances the expression of hair-related keratins in hair follicle keratinocytes, as well as the expression of hair growth-promoting genes in dermal papilla cells. Moreover, the melanin content in UVA-exposed epidermal melanocytes was reduced upon exposure to WS Biotin.

Conclusion: In this work, a novel form of encapsulated biotin, WS Biotin, was developed in order to improve the water solubility of free biotin and was found to be effective for cosmetic use in both hair and skin applications.

KEYWORDS

cell culture, formulation, genetic analysis, hair growth, keratin, melanin

Résumé

Objectif: Développer et évaluer l'efficacité de la WS Biotin, une nouvelle forme hydrosoluble de D-biotine, à usage cosmétique.

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Méthodes: Une nouveau format gélules de D-biotine a été développé dans le but d'améliorer la propriété d'hydrosolubilité de la biotine. Ce nouveau format de gélules de biotine a été caractérisé pour ses propriétés physicochimiques : taille des particules, teneur en D-biotine et solubilité dans l'eau. En outre, des tests *in vitro* de prolifération et d'expression génique en culture cellulaire ont été réalisés pour évaluer son efficacité à favoriser la croissance des cheveux, un test ELISA a été réalisé pour la kératinisation des cheveux et la propriété d'éclaircissement de la peau a été testée en analysant la teneur en mélanine intracellulaire.

Résultats: Les microgélules de WS Biotin développées présentent une plage de tailles de particules de 2 à 30 micromètres avec une teneur en biotine D d'environ 50 % (p/p). L'hydrosolubilité de WS Biotin s'est avérée 20 fois plus élevée que celle de la biotine libre. Les résultats *in vitro* obtenus ont indiqué que WS Biotin améliorait l'expression des kératines capillaires dans les kératinocytes des follicules pileux, ainsi que l'expression des gènes favorisant la croissance dans les cellules papillaires dermiques. En outre, la teneur en mélanine dans les mélanocytes épidermiques exposés aux UVA a été réduite lors de l'exposition à WS Biotin.

Conclusion: Dans ce travail, une nouvelle forme de biotine en gélule, WS Biotin, a été développée afin d'améliorer l'hydrosolubilité de la biotine libre et s'est avérée efficace pour une utilisation cosmétique dans les applications capillaires et cutanées.

INTRODUCTION

Biotin, a.k.a. vitamin B7 or H, serves as a co-factor with key relevance for the function of several metabolic routes in humans. Chemically, it is a bicyclic compound consisting of an ureido group bound to a tetrahydrothiophene group, the latter presenting a valeric acid moiety attached. Among the several stereoisomers of this compound, only D-Biotin displays biological activity [1]. In particular, it needs to be covalently bound to five different carboxylases involved in fatty acid synthesis, amino acid catabolism and gluconeogenesis, to ensure their proper function [2]. Apart from being a co-factor for metabolic enzymes, several reports have revealed its role as a transcriptional regulator for their coding genes. As mammals lack the ability for biotin biosynthesis, they depend on both dietary intake and bacterial origin biosynthesis of this vitamin in the intestinal lumen to supply their demands [1, 3].

The physiological relevance of biotin is mainly revealed by the pathological manifestations of its deficiency. Although uncommon in humans, biotin deficiency leads to a wide range of symptoms involving nervous system, skin and respiratory apparatus [3, 4]. In this regard, some case reports support the beneficial effects of biotin supplementation in hair and nail disorders derived from inherited or acquired biotin deficiency [4]. Nonetheless, so far there is scarce scientific evidence showing the potential benefits of oral biotin supplementation in healthy subjects, except a recent report describing improvements in hair fall and hair density after 6 weeks of administration in individuals with unknown status of biotin deficiency [5]. Despite the molecular events which mediate the potential beneficial effects of biotin are yet to be elucidated, a great number of physicians recommend it for strengthening hair and nails and promoting its growth [6].

Biotin, a white crystalline powder, is very slightly soluble in both water and other pharmaceutically acceptable solvents [7], thus resulting in low bioavailability and reduced functionality.

This poor solubility corresponds to low, and unpredictable bioavailability, making consistent and reliable biotin delivery challenging, and at times impossible, through the natural diet and existing supplementation regimens.

Thus, there is a long-standing and unmet need for biotin compositions to provide enhanced solubility and bioavailability, to facilitate consistent reliable delivery of therapeutic quantities of biotin.

Although biotin has long been used in many hair and skin care formulations, not all formulation strategies for improved water solubility are equally efficacious [8]. In fact, the scarce clinical studies addressing the efficacy of this molecule have been conducted under an oral administration regimen, which might preclude a proper

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evaluation of its action in peripheral tissues such as skin, due to lack of bioavailability. Therefore, scientific evaluation of topical formulations containing biotin would greatly help to resolve the issue of its cosmetic efficacy. For example, biotin may be administered with a cyclodextrin, and dissolved in a small amount of aqueous ammonia [9]. Biotin may also be provided in composition with lactose or amino acid, as an alkalolamine salt [10–12], or as an ester or amide biotin prodrug [13].

On the other hand, studies that shed light on the cell and molecular events influenced by the presence of this molecule are deeply needed. For this reason, and in order to ensure that a critical concentration of biotin in the target area can be achieved upon topical application, the aim of the present work is to design a new encapsulated form of D-Biotin (WS Biotin) and evaluate its efficacy at the cellular and molecular level in different skin cell types, to address its potential use in cosmetic products.

MATERIALS AND METHODS

Preparation of WS Biotin

Seventy-six grams of desalted water were weighted following the addition of 3.20 g of PVP (Kollidon®30, BASF). This water solution was well stirred until reaching homogeneous solution. Then, 6.4 g of biotin (NutriFood) was added to the water solution, followed by the addition of 3.20 g of Octanoic acid (Sigma-Aldrich) while mixing until homogeneous appearance. Following, the pH of the solution was adjusted to a range of 5–7.5 by the addition of NaOH. The obtained solution was subsequently dried using a mini spray dryer Buchi B-290 (Buchi) under the following conditions: inlet air temperature (150°C), feed flow rate (5 mL/min) and blower speed (100%). The obtained powder of microcapsules was sifted by a 200-micron sifter.

Determination of D-Biotin content, entrapment efficacy and yield

D-Biotin was quantified by reverse-phase HPLC using a JASCO HPLC UV-975 system equipped with a variable ultraviolet detector (JASCO Analytical Instruments). Separations were carried out using LiChrospher® 100 RP-18 end-capped column (5 nm, 250 cm*4 mm I.D), with an isocratic mobile phase containing 30% (v/v) Methanol, 30 mM phosphoric buffer with pH 2, at a flow rate of 1.0 mL/min and oven temperature of 30°C. D-Biotin was detected at 210 nm.

The D-Biotin content (% w/w) in the microcapsules was defined as the result of the division of the measured D-Biotin content in microcapsules by microcapsule powder weight.

The entrapment efficiency level was calculated using the following equation:

$$\text{Encapsulation efficiency} = \text{estimated content} / \text{theoretical content} \times 100$$

Production yield was determined and calculated by - % Yield $= W/Wo \times 100$ where W is the actual weight of microcapsules powder and Wo is the total expected weight (theoretical).

Size and morphological characterization

Microcapsules were analysed for their size and morphology by using scanning electron microscopy (SEM 505, Philips). Specimens for SEM observation were made by mounting the microcapsules directly on SEM grid, using double-side sticking tape and coated with platinum under reduced pressure and then placed in scanning electron microscope.

Solubility test

The water solubility property of WS Biotin powder was measured in distilled water (DW) by adding the WS Biotin powder at 1%, 2% and 4% (equivalent to 0.25%, 1% and 2% of free D-Biotin, respectively) and mixing for 10 s at room temperature. The solubility test was conducted at room temperature. In comparison, 0.1% of D-Biotin powder was dissolved in water by heating the water for 80°C and mixed for 10 min.

Cell culture

Human Hair Follicle Keratinocytes (HHFK, Sciencell, Cat # 2440 lot 34446) were maintained in Keratinocyte Medium (KM, Sciencell, Cat # 2101) supplemented with Keratinocyte Growth Supplement (KGS, Sciencell, Cat # 2152) and passaged when they reached ~80% confluence.

Human Follicle Dermal Papilla Cells (HFDPC, Cell Applications, Cat # 602-05a lot 2598) were maintained in Follicle Dermal Papilla Cell Growth Medium (FDPCGM, Promocell, Cat # 26500) supplemented with Growth Medium SupplementMix (Promocell, Cat # 39625) and passaged when they reached ~80% confluence.

Normal Human Epidermal Melanocytes M2 (NHEM, Promocell, Cat # 12402 lot 455Z009.2) were maintained in Melanocyte Growth Medium M2 (MGM2, Promocell, Cat # 24400) supplemented with Growth Medium M2 SupplementMix (Promocell, Cat # 39420) and passaged when

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they reached ~60% confluence to avoid overgrowth that affected the accumulation of melanin.

Cell viability

HHFK were seeded on Falcon® 96-well plates (Corning) at a density of 8×10^3 cells/well in KM + KGS and allowed to attach to the plastic culture overnight. Subsequently, the seeding medium was removed, and cells were exposed to Biotin WS at doses ranging from 0.001% to 3% (w/v) in KM + KGS for 48 h at 37°C. Next, cells were washed with PBS and incubated with MTT (Fisher Scientific) 0.5 mg/mL in KM for 3 h at 37°C. The absorbance of 570 nm of the DMSO-solubilized crystals was recorded with a multi-well reader.

HFDPC were seeded on Falcon® 96-well plates (Corning) at a density of 1×10^4 cells/well in FDPCGM + SupplementMix and allowed to attach to the plastic culture overnight. Subsequently, the seeding medium was removed, and cells were exposed to Biotin WS at doses ranging from 0.001% to 3% (w/v) in FDPCGM + SupplementMix for 24 h at 37°C. Next, cells were washed with PBS and incubated with MTT (Fisher Scientific) 0.5 mg/mL in FDPCGM for 3 h at 37°C. The absorbance of 570 nm of the DMSO-solubilized crystals was recorded with a multi-well reader.

NHEM were seeded on Falcon® 96-well plates (Corning) at a density of 5×10^3 cells/well in MGM2 + SupplementMix and allowed to attach to the plastic culture overnight. Subsequently, the seeding medium was removed, and cells were exposed to Biotin WS at doses ranging from 0.001% to 3% (w/v) in MGM2 + SupplementMix for 72 h at 37°C. Next, cells were washed with PBS and incubated with MTT (Fisher Scientific) 0.5 mg/mL in MGM2 for 3 h at 37°C. The absorbance of 570 nm of the DMSO-solubilized crystals was recorded with a multi-well reader.

Cell proliferation

HHFK were seeded on Falcon® 96-well plates (Corning) at a density of 5×10^3 cells/well in KM + KGS and allowed to attach to the plastic culture overnight. Subsequently, the seeding medium was removed, and cells were exposed to Biotin WS at different doses, as well as 20 ng/mL human recombinant Epidermal Growth Factor (hrEGF, Sigma) in KM + KGS diluted 20-fold from the recommended concentration for 48 h at 37°C. Next, cells were washed with PBS and incubated with MTT (Fisher Scientific) 0.5 mg/mL in KM for 3 h at 37°C. The absorbance of 570 nm of the DMSO-solubilized crystals was recorded with a multi-well reader.

Quantification of hair keratins

HHFK were seeded on Falcon® 60 mm TC-treated Cell Culture Dishes at 3×10^5 cells/dish in KM + KGS and allowed to attach to the plastic culture overnight. Subsequently, the seeding medium was removed, and cells were exposed to Biotin WS at different doses in KM + KGS for 48 h at 37°C. After treatment, cells were collected and lysed with freeze/thaw cycles at −80°C in PBS + Pierce Protease Inhibitor Cocktail (Thermo Scientific). The soluble fraction was assayed for KRT40 and KRT86 with specific ELISA kits (Abbexa) and the specific protein content was normalized versus the total protein content, determined with the Pierce BCA Protein Assay Kit (Thermo Scientific).

Gene expression of hair growth-related growth factors

HFDPC were seeded on Falcon® 24-well plates (Corning) at 10^5 cells/well in FDPCGM + SupplementMix and allowed to attach to the plastic culture overnight. Subsequently, the seeding medium was removed, and cells were exposed to Biotin WS at different doses in FDPCGM + SupplementMix diluted 20-fold from the recommended concentration for 24 h at 37°C. After treatment, RNA was isolated with the RNeasy Mini Kit (Qiagen), complementary DNA (cDNA) was synthesized with the PrimeScript RT Reagent Kit (Takara Bio Europe) and the gene expression was determined with PrimeTime STD assays (IDT) specifically targeted to *ACTB*, *VEGF*, *IGF1* and *FGF7*. Specific sequences of primers and probes can be found in Table S1. Relative expression was calculated using the $2^{-\Delta\Delta Ct}$ algorithm.

Melanin quantification

NHEM were seeded on Falcon® 24-well plates (Corning) at 10^5 cells/well in MGM2 + SupplementMix and allowed to attach to the plastic culture overnight. Subsequently, the seeding medium was removed, and cells were exposed to Biotin WS at different doses in MGM2 + SupplementMix for 5 days at 37°C. During the first 3 days, cells were subjected to one daily dose of UVA (10 J/cm^2) to stimulate melanogenesis. In parallel, a set of wells remained non-irradiated as a basal control. After the treatment, cells were harvested and pelleted, and melanin was extracted after incubation at 60°C with DMSO 10% in NaOH. The melanin content was estimated through spectrophotometric measurements at 405 nm and normalized to cell viability measured by the MTT assay, as outlined above.

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RESULTS

Determination of D-Biotin content, entrapment efficacy and yield

The well-established microcapsules manufacturing method yielded microcapsules containing 46.1% (w/w) D-Biotin with a production yield ranging between 80 and 85%. The entrapment efficacy ranged between 85% and 90%.

WS Biotin powder characterization

The WS Biotin appears as a white powder (data not shown). Particle morphology and size were analysed by scanning electron microscopy (SEM), which revealed that the powder contains spherical particles as with a size range of 2–30 μm (Figure 1).

WS Biotin water solubility test

The tests, performed at different concentrations of the WS Biotin water solution, revealed that this novel form of D-Biotin enhances its solubility in water. As shown in Figure 2, WS Biotin increased the solubility of D-Biotin up to 40 times when compared to the free form of the D-Biotin molecule without any need for alcohol as a solubilizer. Likewise, no recrystallization of D-Biotin was observed after cooling the solutions to room temperature. In contrast, dissolving 0.1% of free D-Biotin required a much higher temperature and longer mixing time, and recrystallization phenomena was observed when the solution was brought to room temperature.

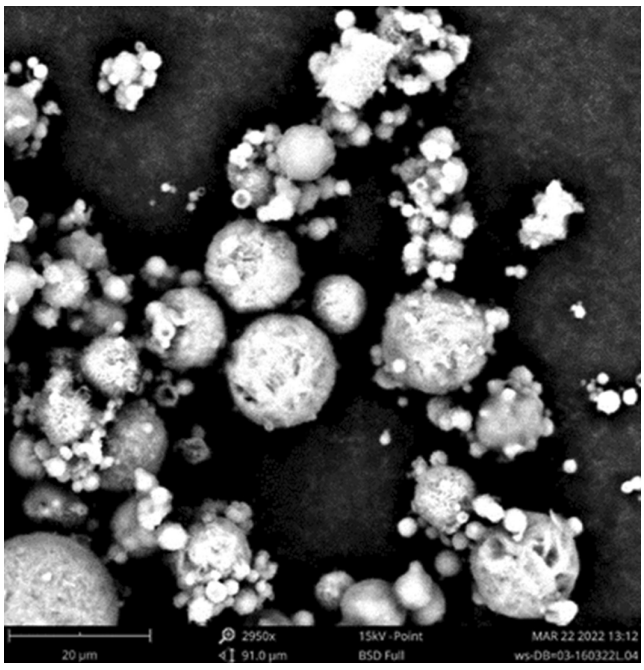


FIGURE 1 The encapsulation of D-Biotin into WS Biotin yields multiple size particles. SEM micrograph of the WS Biotin powder (as microcapsules) obtained after drying.

Characterization of the cytotoxicity profile of WS Biotin in different in vitro models

With the aim to elucidate the possible beneficial effects of WS Biotin in relevant cosmetic attributes at the molecular level, three in vitro platforms were selected, namely HHFK, HFDPC and NHEM. To rationally select the concentrations of the WS Biotin water solution to be assayed in functional assays, a cytotoxicity test was conducted in the first place.

In this regard, HHFK exposed for 48 h to WS Biotin in culture medium at doses between 0.001% and 0.1% (w/v) did not undergo relevant changes in cell viability. In contrast, increasing the concentration of this WS Biotin water solution in the culture medium exerted detrimental effects on the cells: 0.3% was cytostatic, as demonstrated by a reduction of 38% in cell viability versus untreated control, while 1% and 3% were markedly cytotoxic for this cell type, with decreases in cell viability of nearly 100% versus untreated control (Figure 3a). Based on these results, a dose range below 0.1% was chosen for subsequent functional assays.

In a similar approach, HFDPCs were cultured in the presence of WS Biotin for 24 h, to reproduce the exposure time chosen for the further study of gene expression. Under these conditions, the WS Biotin water solution was moderately cytostatic at 0.3%, with cells exposed to this dose displaying cell viability values 14.4% below untreated control; mildly cytotoxic at 1%, which decreased cell viability by 28.2%, and sharply cytotoxic at 3%, which reduced viability to less than 5% of the untreated control (Figure 3b). Thus, 0.1% and 0.01% were the doses selected for the further study of gene expression.

Finally, NHEM were subjected to long-term exposure to WS Biotin, as this treatment schedule would be used to test the effectiveness of this sample in preventing melanin accumulation, which takes several days. Interestingly, all the concentrations included within the range 0.001–0.3% stopped the growth of NHEM, with cell viability values very similar to those of cells seeded before treatment (control 0 h). In agreement with findings in HHFK and HFDPC, marked cytotoxicity was induced by WS Biotin at 1% and 3% (Figure 3c). Consequently, 0.01% and 0.1% were the doses selected to evaluate the anti-melanogenic potential of the WS Biotin water solution.

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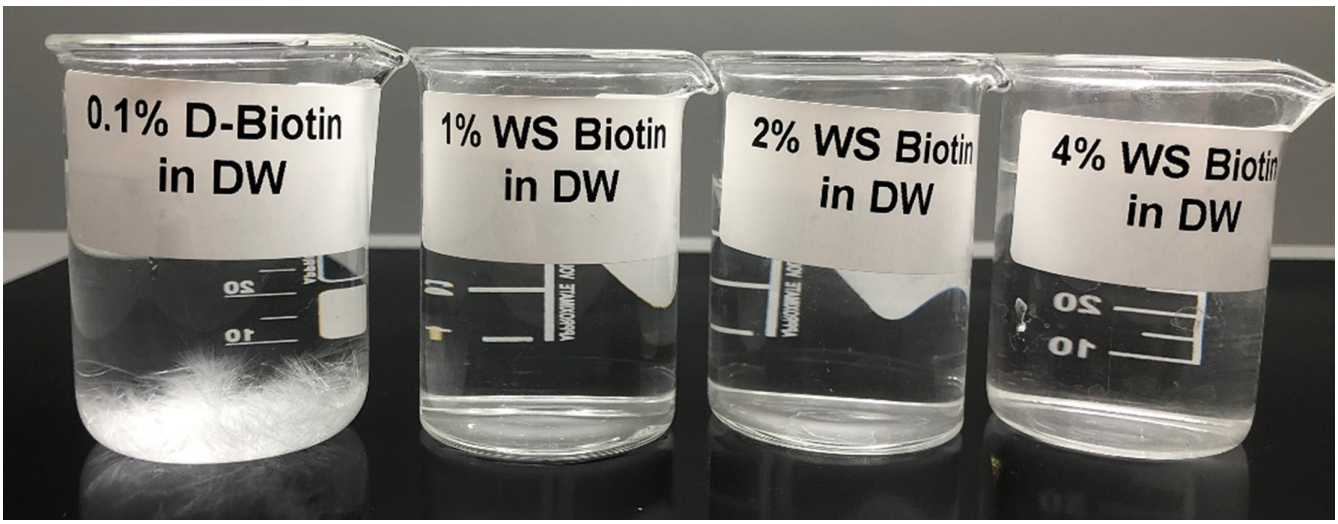


FIGURE 2 WS Biotin presents much higher solubility than free D-Biotin. Images of WS Biotin solutions in DW at different concentrations of 1%, 2% and 4% (equivalent to 0.5%, 1% and 2% free D-Biotin, respectively) as compared to solution of 0.1% D-Biotin (after cooling down).

Influence of biotin WS in the proliferation of hair follicle keratinocytes

Hair follicle keratinocytes (HFKs) located in the matrix are highly proliferative and support the elongation of the hair shaft throughout the hair cycle [14]. To address the possible beneficial use of Biotin WS in hair shaft elongation, HHFK were cultured in the presence of this WS Biotin water solution for 96 h in a supplement-reduced medium. In parallel, EGF, a well-known mitogen [15], was used as a positive control.

As expected, incubation with EGF in a supplement-reduced medium enhanced HHFK proliferation as shown by a ~73% increase in cell viability versus untreated cells. A milder effect was seen upon exposure to WS Biotin, with a non-statistically significant increase in cell viability by >15% at 0.0001% and 0.1% (Figure 4). All in all, these results may suggest a subtle, yet beneficial effect of biotin supplementation in promoting hair shaft elongation. Nevertheless, complementary proliferation assays (e.g. BrdU incorporation) would be helpful to rule out the possibility that these changes are derived from technical background.

WS Biotin promotes the expression of hair shaft keratins

In an attempt to assess the capacity of WS Biotin to enhance the biomechanical properties of hair, the levels of two different hair-associated keratins were studied. Two representative members of type I and type II families of hair fibre keratins, namely KRT40 and KRT85 [16] were selected for this purpose.

In this regard, intracellular protein extracts from HHFK treated with WS Biotin for 48 h displayed a dose-dependent increase in the levels of KRT40. The doses 0.001% and 0.01% increased the relative levels of this protein by 7.0% and 19.3%, respectively, although differences did not reach statistical significance. Importantly, there was a marked and statistically significant increase in the relative abundance of this protein versus the total protein content by 80.6% versus untreated control (Figure 5a). Similarly, KRT85 levels were increased by 11.1% and 21.4% at 0.001% and 0.01, respectively, in a non-statistically significant manner, whereas 0.1% WS Biotin dramatically boosted the expression of this protein by 81.2% versus the untreated control (Figure 5b). Taken together, these data would support the amelioration of hair shaft strength upon exposure to the studied WS Biotin water solution.

Modulation of hair growth-related genes by WS Biotin

Different growth factors are known to positively regulate the hair cycle, namely Insulin-like Growth Factor 1 (IGF1), Vascular Endothelial Growth Factor (VEGF) and Fibroblast Growth Factor 7 (FGF7, a.k.a. KGF) [17–19]. For this reason, the expression of their coding genes was evaluated in HFDPC exposed to WS for 24 h. As shown in Figure 6a, *VEGF* was dose-dependently upregulated by the tested WS Biotin water solution, with its expression being increased by 0.6-fold versus untreated control in a

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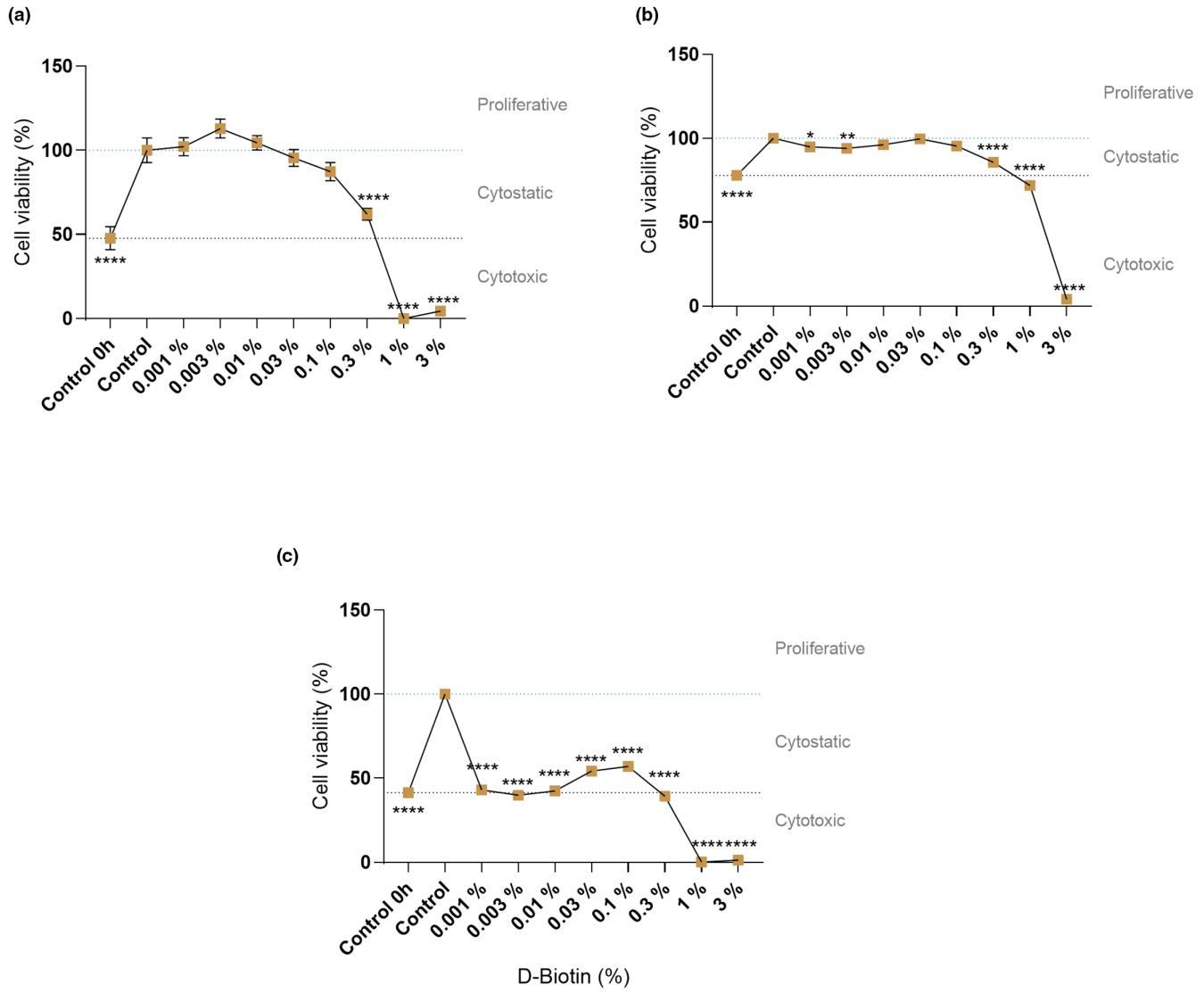


FIGURE 3 Cytotoxicity profile of WS Biotin in different in vitro systems. Relative cell viability values (calculated versus untreated control) of HHFK treated for 48 h (a), HFDPC treated for 24 h (b) and NHEM treated for 72 h (c) with WS Biotin, obtained by the MTT assay.

statistically significant manner upon exposure to WS Biotin at 0.1%. Consistently, treatment of HFDPC to this same dose triggered a statistically significant upregulation of *FGF7* by 1.3-fold versus untreated control (Figure 6b). On the other hand, *IGF1* underwent the greatest gene expression improvement at the same dose, with a statistically significant increase of 1.7-fold in its expression (Figure 6c). In summary, these data, together with the positive trend shown in Figure 4, point towards a hair maintenance-promoting potential of WS Biotin at the molecular level.

Whitening potential of WS Biotin

Despite the protective roles of melanin, an excess of skin pigmentation is the underlying cause of several pathologies, and some cultures regard light skin tone as a sign of youth and beauty. For this reason, cosmetic formulations with whitening potential are greatly pursued, with an especially important source in natural products [20–22]. To test the potential of WS Biotin as a skin lightener in vitro, cultured NHEM stimulated with UVA radiation were exposed to this WS Biotin water solution. First, the tanning effect of UVA was confirmed, as shown by the normalized melanin levels in non-irradiated cells, which were 32.0% lower than those of cells receiving UVA at 30 J/cm^2. Importantly, WS Biotin at 0.01% and 0.1% diluted in complete cell culture medium triggered a dose-dependent decrease in melanin levels by 8.2% and 18.4%, respectively, versus untreated control ($p > 0.05$; Figure 7). Based on this data, a skin-lightening effect might be attributed to WS Biotin.

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DISCUSSION

The use of biotin for the improvement of skin conditions has long been known, although few evidence is available on the benefits of this molecule in healthy individuals, as well as at the cellular and molecular level. With the aim of shedding the light on both issues, in this work, a series of functional studies using the novel WS Biotin, encapsulated biotin for improved water solubility, have been conducted in different in vitro models that recapitulate relevant aspects of skincare, such as hair growth, hair strength and pigmentation.

Prior to the assessment of functional effects, the cytotoxicity profile of WS Biotin was studied in HFDPC, HHFK and NHEM, with variable profiles, being NHEM the most affected cell type by the exposure to this form of Biotin. It is important to stress that different exposure times were set for each cell type that was as close as possible to the incubation with the product for functional assays. In this regard, a standard exposure time for the three cell types would better clarify whether differences in toxicity really exist between them. As far as the authors' knowledge, there are no similar works addressing the potential cytotoxicity of this compound in either animal or human-derived cell lines.

When HHFK were treated with WS Biotin at sub-cytotoxic doses, an increasing trend in proliferation was observed, although the results are not conclusive due to the absence of statistical significance and might arise from technical background. Previous work ruled out the possibility of a proliferative effect mediated by biotin in follicular keratinocytes at doses around 10^{-5} M for 36 h [23]. In the present work, doses between 10^{-6} and 10^{-3} M were applied in HHFK, including the highest dose used by Limat et al., for 96 h. Thus, further research is needed to clarify whether a longer exposure to biotin might be beneficial for HHFK proliferation. In this regard, a positive correlation between histone biotinylation and cell proliferation has been described, even though it is not clear which of them is the cause and the consequence [24]. Further research is needed to clarify this issue.

The formation of the hair shaft involves the progressive expression of different keratins in a fine-tuned manner [25]. Among them, Keratin 85 (KRT85) stands for its widespread localization in the matrix, precortex, cortex and cuticle, as well as the clinical manifestations in individuals with mutations in its coding gene [26]. On the other hand, Keratin 40 (KRT40) is a key type I keratin whose expression is crucial to achieving a full differentiation of hair shaft [27]. In this study, exposure of HHFK to WS Biotin greatly enhanced the expression of both proteins, thus supporting the potential to promote the acquisition of appropriate biomechanical properties, through the stimulation of proteins involved in the keratinization of the hair shaft. Although there is a popular idea that biotin supports keratin biosynthesis, none of the enzymes that utilize it is involved in protein or amino acid synthesis, rather in its catabolism (malonyl-CoA carboxylase, involved in the catabolism of leucine) [24]. Previous work in biotin-deficient animal models supports the positive influence of biotin intake on the overall protein synthesis [28], but such studies have not been conducted in healthy individuals.

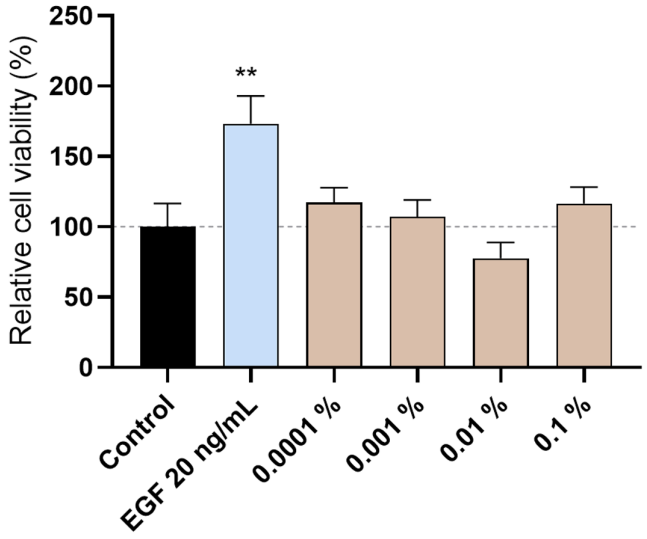


FIGURE 4 The exposure of HHFK to WS Biotin may promote follicular keratinocyte proliferation. Relative cell viability of HHFK in supplement-reduced culture media exposed to WS Biotin at different concentrations for 96 h.

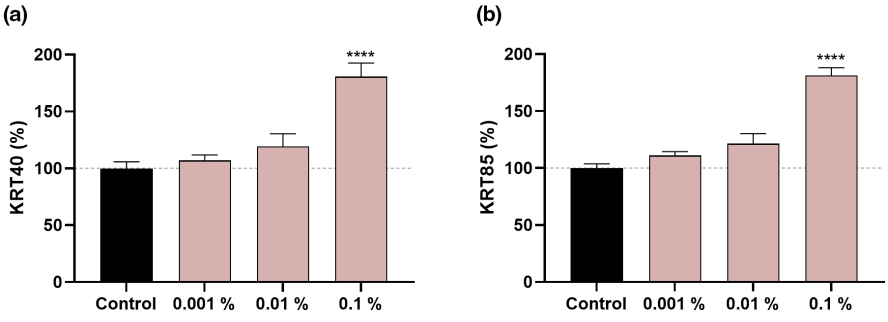


FIGURE 5 The application of WS Biotin the production of relevant keratins in follicular keratinocytes. Relative levels of KRT40 (a) and KRT86 (b) normalized to total protein content in cell lysates from HHFK treated for 48 h with WS Biotin, determined by ELISA.

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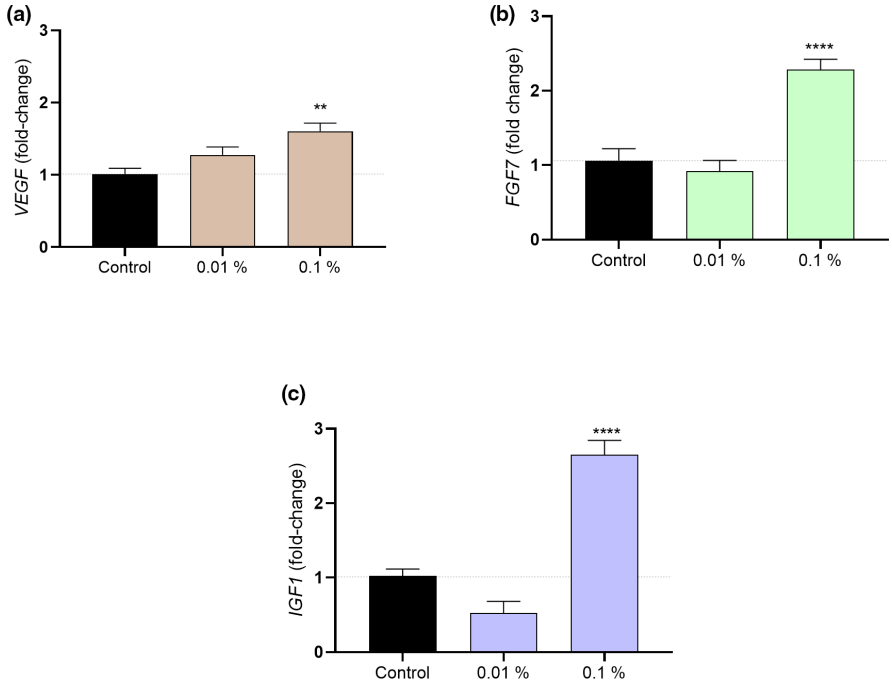


FIGURE 6 WS Biotin promotes the expression of hair growth-related genes. Relative gene expression values of *VEGF* (a), *FGF7* (b) and *IGF1* (c) in HFDPCs exposed to WS Biotin at different concentrations in supplement-reduced culture medium, determined by qPCR.

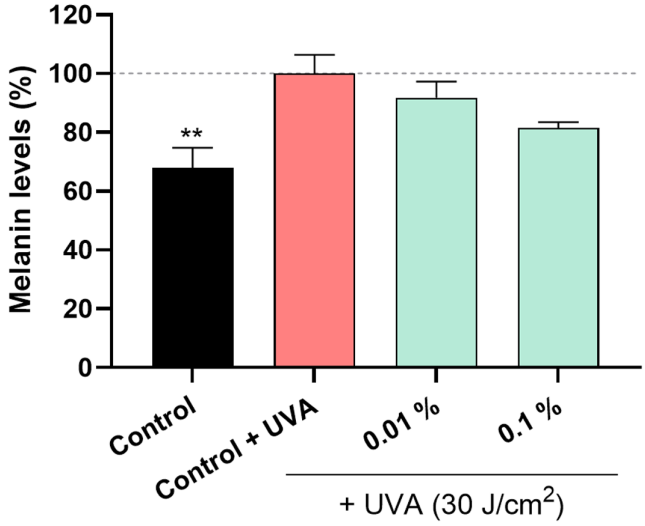


FIGURE 7 Skin-lightening potential of WS Biotin. Relative levels of intracellular melanin in NHEM exposed to WS Biotin for 6 days and simultaneously subjected to irradiation with UVA (cumulative dose: 30 J/cm^2) for the first 3 days. Melanin levels were normalized to cell viability at the end of the treatment.

Therefore, to the authors' knowledge, the present work is the first to provide evidence substantiating the popular idea that biotin supplementation is beneficial for hair keratin synthesis.

The dermal papilla is a subepidermal structure supporting the elongation of the hair shaft throughout the hair cycle. Therefore, the potential benefits of biotin supplementation in hair growth would be expected to be mediated by the stimulation of this structure. In this regard, recent work supports the positive impact of biotin in hair growth and maintenance [5, 29], with the mechanism of action remaining unexplored. The results presented in this study not only reinforce this notion but also shed light on the molecular events underlying the hair growth-promoting ability of biotin, which would be mediated by the upregulation of *VEGF*, *FGF7* and *IGF1*, in HFDPCs.

Biotin has already been included in proprietary formulations as a skin-lightening agent in vivo [30], even in winter months, where the incidence of UVR is weaker. The results here presented not only support this idea but also show that the anti-pigmentation potential of this molecule is displayed even in the presence of a strong skin-tanning stimulus such as UVA radiation, with reduction percentages in melanin content relevant from a cosmetic point of view.

Overall, it is important to highlight that the doses used in this study greatly exceed the reported concentrations of biotin in human blood (0.1–0.8 ng/mL) [31]. Thus, the results presented herein support the development of enhanced vehiculization strategies capable of delivering biotin in amounts sufficient to trigger the above-described effects in the complex system of human skin.

In conclusion, the results presented in this work support the beneficial use of WS Biotin for cosmetic purposes at multiple levels and preliminary delineates the cellular and molecular targets underlying these effects in hair growth and reinforcement, as well as a skin lightening agent. The beneficial effect of WS Biotin is due to its improved solubility in water which results in increased bioavailability and improved efficacy.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

ORCID

Alejandro Pérez-Fernández https://orcid.org/0000-0001-7862-7952

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SUPPORTING INFORMATION

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