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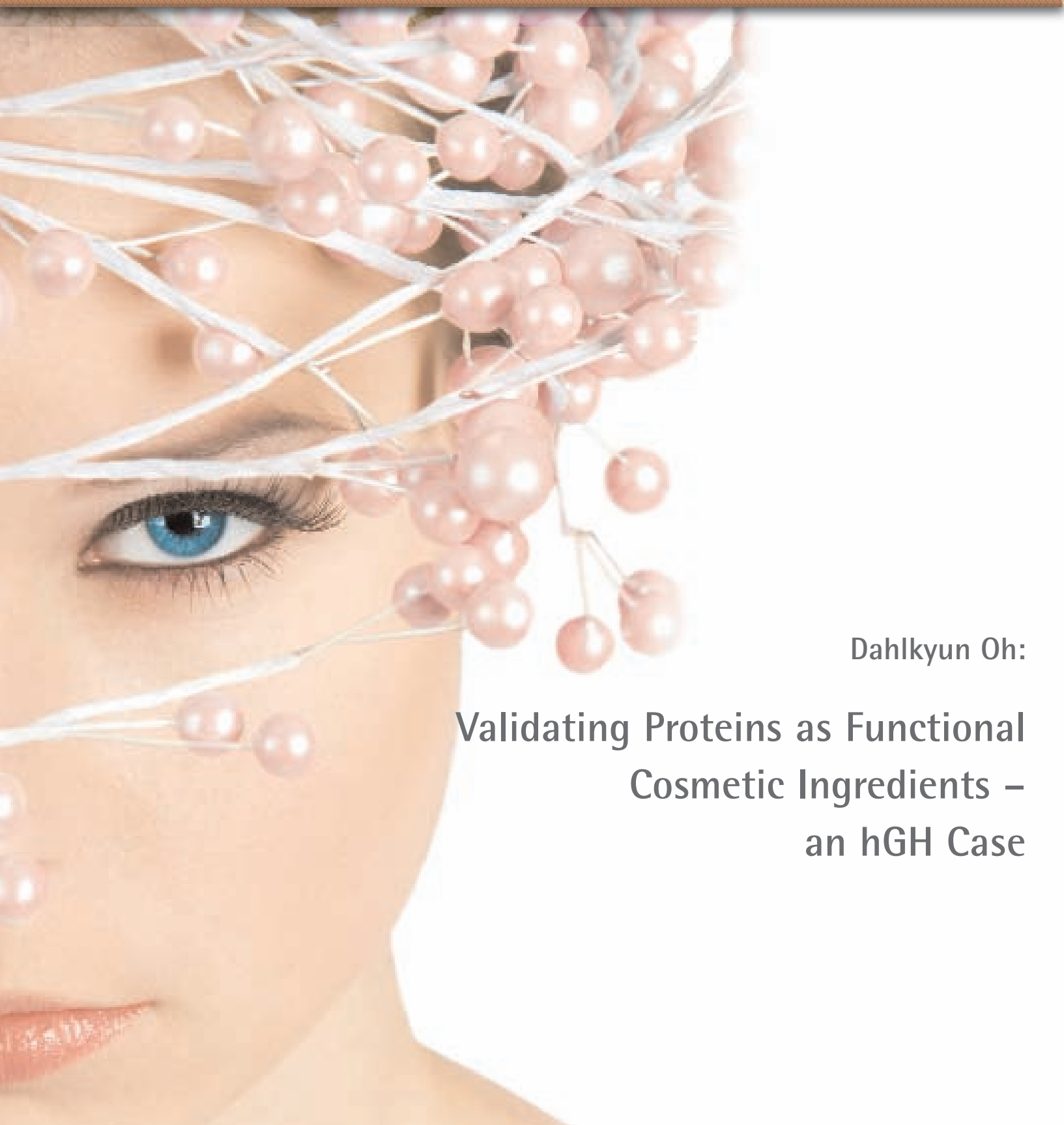


Dahlkyun Oh:

Validating Proteins as Functional Cosmetic Ingredients – an hGH Case

Dahlkyun Oh*

Validating Proteins as Functional Cosmetic Ingredients – an hGH Case

Abstract

Traditionally, proteins were not considered as qualified candidates for cosmetic active ingredients mainly due to 1) their intrinsic labile nature in an aqueous solution and 2) their formidable size barriers in reaching viable skin layers.
Specifically targeting cells of the hair follicle through encapsulating a protein ingredient inside a liposome, an effective cosmetic outcome as well as an efficient protein delivery to critical cells of the skin can be attained. Presented here is a summary of efforts to rationalize what was observed regarding the cosmetic application of a liposome-encapsulated human growth hormone (hGH).

■ Proteins as Cosmetic Ingredients

Does a protein cosmetic ingredient make sense to you?
Proteins are in general hydrophilic or hydrophobic macro-biomolecules composed of more than 20 amino acids with approximate molecular weight (m.w.) of 2000 dalton (2kd) or higher. Oligopeptides, on the other hand, normally consists of less than 20 amino acids. In the cosmetic field, when people talk about peptides, they refer to oligopeptides, often composed of fewer than 10 amino acids. Traditionally, macromolecules with m.w. of more than 500 dalton were considered difficult to pass through the skin epidermis (1). Even with the help of chemical penetration enhancers, macromolecules with m.w. more than 2000 dalton were considered practically implausible to permeate through the skin. Therefore, when people develop peptides as cosmetic ingredients, they consciously try to adopt an oligopeptide consisting of less than 10 amino acids (m.w. roughly about 1100 dalton), if possible, to enable it to reach the skin dermis. Given these notions, it's not surprising that people puzzle over an attempt to use a protein as large as hGH (human growth hormone) with 191 amino acids (m.w., ~22000 dalton) **(Fig. 1)** as an active cosmetic ingredient on the intact skin and wonder about whatever efficacies can come out of its application. What's more unexpected was, despite overwhelming skepticism, the experimental outcome of topical application of liposome-encapsulated hGH on the intact skin *in vivo*, both in human and mouse, that clearly showed its efficacies on the skin as diverse as acne alleviation **(Fig. 2)**, sunlight-caused dark spots removal **(Fig. 3)** in addition to skin tone improvement **(Table 1)** in human applications and UV-induced wrinkle removal **(Fig. 4)** in mouse experiments. To reconcile these observations with the long established dogma, some kind of as yet unexplored logical explanation should be rendered. This assay is an attempt to encompass and rationalize these two seemingly contradicting experimental observations – one, impermeability of the skin to a macro-biomolecule like hGH and the other, experimental *in vivo* efficacies of hGH on the intact skin - mustering some relevant scientific reports, and also give some perspectives on future selections and qualifications of protein cosmetic ingredients.

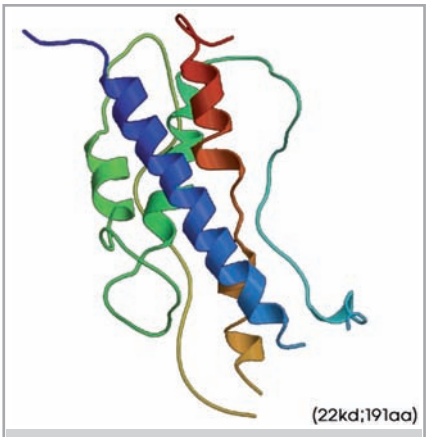


Fig. 1 Structure of human growth hormone (adopted from RCSB Protein Data Bank)

■ About Human Growth Hormone

Human growth hormone is a hydrophilic polypeptide composed of 191 amino acids and has approximate m.w. of 22 kd. This protein has two pairs of disulfide bonds and extensive α-helical structural motifs **(Fig. 1)**. Together they confer compactness and sturdiness on hGH molecules. However, although hGH can be considered as a stable, tough protein by the norms of the protein world, it is of course

subject to various physical, chemical, and biological deteriorations as most other proteins are. It needs protection from oxidative damage, conformational changes, enzymatic degradation, aggregation, precipitation, etc.. Moreover, though hGH can be stored more than 2 years through freeze-drying with the help of stabilizing disaccharide like lactose or sucrose, once it's dissolved in an aqueous solution, hGH becomes labile and susceptible to a variety of assaults just mentioned above; thereby limiting its application as a cosmetic ingredient, let alone its efficacies on the intact skin. Thus, it is imperative to first find a protective carrier for hGH that enable hGH to reach a target tissue layer and at the same time can shield hGH from protease attacks and adverse conditions on the way.

HGH is originally produced in the pituitary gland in the forebrain and secreted into the blood for circulation. Its half-life in the blood as a monomer is as short as 15 min or so (2) and its major target organ is the liver where hGH binds to hGH receptors on liver cells. Classically accepted notion is that hGH transmits a signal to them so that the vast majority of its effects on the body can be realized through the secondary hormone effector IGF-1 (Insulin-like-growth factor-1) that is produced by the liver in response to hGH action to the liver. Now this notion is under serious re-evaluation as hGH seems to possess its authentic functions in various tissues of the body apart from IGF-1-mediated ones (3).

Nowadays, hGH can be produced efficiently and safely through microbial fermentation and subsequent purification, owing to advances of biotechnology. When administered as an injection, hGH was claimed to exert as diverse effects to the body as stimulating bone and muscle growth, helping develop secondary sexual characteristics in the youth (4), and increasing libido, reducing abdominal fat, removing plaques from blood vessels (5), fortifying body's immunity, expanding cardiovascular and respiratory outputs, and even improving skin tone and elasticity in the adult (6). Unsubstantiated claims such as an improved vision, hair sprouting, and hair-color darkening have been advanced as well (ibid.). It is these sorts of seemingly rejuvenating or

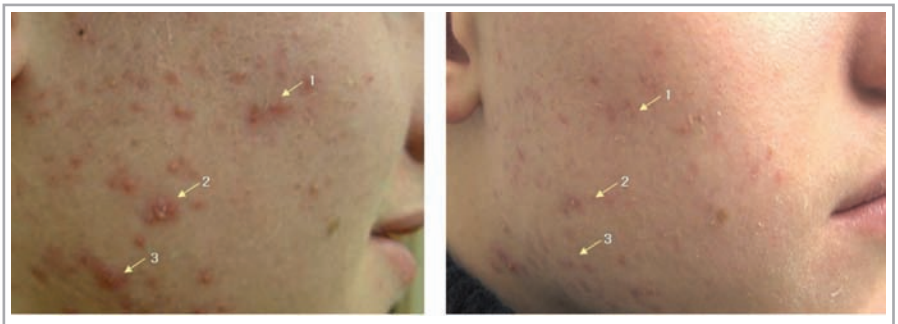


Fig. 2 Anti-acne effect of a NL-hGH solution

Fig. 3 Whitening effect of a NL-hGH formulation

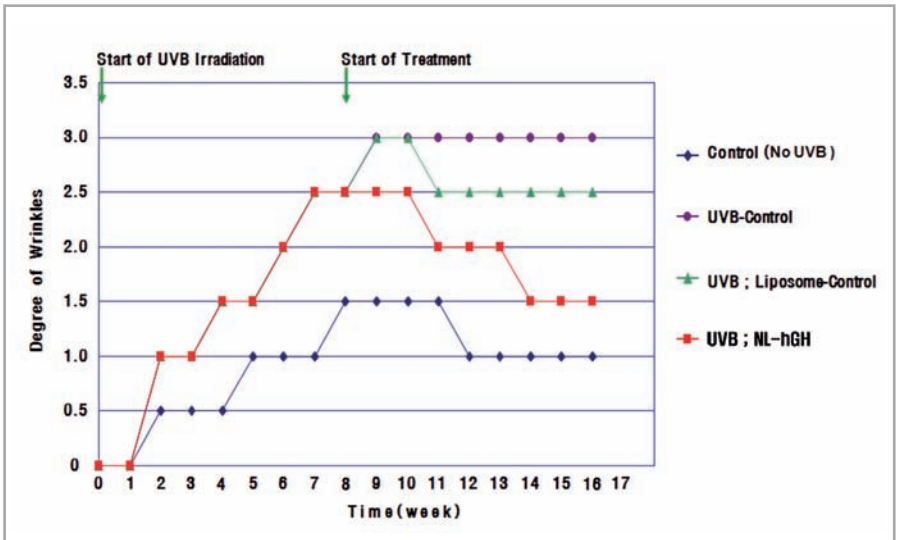


Fig. 4 Removal of UV-induced wrinkles by a NL-hGH solution

A. Panelist Questionnaire

Panelist Questionnaire — **Conducted by: Cantor Research Labs, NY, USA**

Statement	4 Week					8 Week				
	Somewhat Agree	Strongly Agree	Somewhat Disagree	Strongly Disagree	Overall Agreement	Somewhat Agree	Strongly Agree	Somewhat Disagree	Strongly Disagree	Overall Agreement
Significantly reduces the appearance of fine lines and wrinkles	5	3	8	0	50%	9	6	0	1	94%
Significantly reduces roughness and dryness	6	4	5	1	63%	5	11	0	0	100%
Significantly diminishes the appearance of age spots, freckles, and skin discolorations	6	1	9	0	44%	9	3	3	1	75%
Significantly lightens the skin	7	1	8	0	50%	9	4	2	1	81%
Significantly improves skin's softness and smoothness	9	6	1	0	94%	3	13	0	0	100%
Significantly improves skin's radiance, tone, and clarity	9	3	4	0	75%	4	9	3	0	81%
Significantly improves skin's firmness, tightness, and elasticity	8	4	4	0	75%	8	6	2	0	88%
Significantly moisturizes the skin	6	7	3	0	81%	3	12	1	0	94%
Significantly improves skin's overall appearance	6	6	4	0	75%	6	9	1	0	94%

B. Spectrophotometer Reading, Cutometer Reading, Analysis of Replica

Test Method		SPECTROPHOTOMETER READINGS (Statistically Significant Critical Value =1.7530)				CUTOMETER READINGS (SSCV = 1.7530)					ANALYSIS OF REPLICAS (SSVC=1.7709)			
Test Items		L values (SCI) of Hyper-Pigmented Spot	L values (SCE) of Hyper-Pigmented Spot	L values (SCI) of Surrounding Skin	L values (SCE) of Surrounding Skin	R2	R5	R6	R7	F2	RA North-South	RA East-West	Shadows North-South	Shadows East-West
Day0	Mean	53.33	53.19	61.45	61.31	0.5584	0.4755	0.6203	0.2958	0.2654	16.49	14.26	29.36	27.41
Week 4	Mean	54.10	53.97	61.19	61.04	0.5948	0.4816	0.5745	0.3059	0.2595	14.50	13.20	29.09	26.96
	%Difference	1.45	1.47	-0.42	-0.44	6.52	1.27	-7.37	3.44	-2.22	-12.04	-7.41	-0.92	-1.64
	t-STAT	-2.31	-2.26	0.39	0.42	-1.48	-0.19	1.17	-0.55	0.21	1.75	1.60	0.14	0.30
	Statistical Significance	Significant	Significant	Significant	Significant	Significant	Significant	Significant	Significant	Significant	Significant	Significant	Significant	Significant
Week 8	Mean	54.84	54.69	61.78	61.62	0.6152	0.5721	0.7481	0.3263	0.2287	15.33	14.09	27.92	27.01
	%Difference	2.84	2.83	0.54	0.51	10.19	20.30	20.61	10.33	-13.85	-7.02	-1.20	-4.91	-1.48
	t-STAT	-3.43	-3.48	-1.25	-1.16	-2.26	-2.51	-2.44	-1.73	1.27	0.83	0.27	0.71	0.24
	Statistical Significance	Significant	Significant	Significant	Significant	Significant	Significant	Significant	Significant	Significant	Significant	Significant	Significant	Significant
Remarks		Females between 35 and 65. L values= Lightness Values SCI= Specular Component Included SCE= Specular Component Excluded				Gross Elasticity=ability of redeformation	Net Elasticity. The closer to 1 represents the more elastic.	Viscoelasticity. The smaller the value, the higher the elasticity	Elasticity The closer the value is to 1, the more elastic the skin is.	the smaller the value, the firmer the skin.	Shadowed area of deviation above and below the averaged center line of the wrinkled(shadowed) area.		Shadowed region associated with wrinkles	

Table 1 Efficacy Test Results of a Nanolipo-hGH Formulation (through 3LAB™ »h«-Serum)

aging-defying effects of hGH subcutaneous injections especially on the elderly that elicited a bonanza of interests on hGH and an avalanche of quasi-hGH dietary supplements luring the gullible.
Alhough hGH has enjoyed such a wide recognition, its cosmetic application and the initial observations related to its efficacies were quite serendipitous. The followings are summary reports of follow-up experiments engendered by those observations.

Some Issues Around hGH as a Cosmetic Ingredient

1. Stability issue

The weakest point of a protein cosmetic ingredient is its labile nature such as its intrinsic propensity for denaturation, degradation, or aggregation in a solution state. Thus, if hGH can be used as a cosmetic ingredient, it should overcome minimum stability requirements set for a cosmetic product. HGH is prone to aggregation and degradation in solution, but much of it can be overcome by encapsulating hGH inside a liposome. The possibility of hGH either trapped in the phospholipid hydrophobic tail portion of a phospholipid bilayer or attached to the liposome surface rather than encapsulated inside the aqueous core has been ruled out through relevant experiments (*Kyunyoung Lee* & *Dahlkyun Oh*, unpublished results). Surprisingly, liposome-encapsulated hGH could last a year and still retain about 70% of its original bioactivity at room temperature and about 85% at 4 °C **(Fig. 5)**. It should be noted that, although some hGH molecules in solution undergo oxidative cleavage resulting in two fragments, yet those cleaved fragments are still held together by disulfide bonds and still maintain bioactivity in cell-based bioassays (*Kyunyoung Lee* & *Dahlkyun Oh*, unpublished results). It should also be noted that non-encapsulated hGH degrades much faster and the rate of hGH degradation seems closely related to the purity of and contaminants in hGH preparations. Of course, the number of surviving microbes contaminating hGH-containing solution can affect the shelf-life of the solution for sure.

2. Delivery Issue

It's well known that hydrophilic macromolecular proteins like hGH can not penetrate the skin epidermis by itself due to hydrophobic nature of the epidermal keratin layer. Previous attempts to deliver proteins deeper into the dermis of the skin are scarce and grossly unsuccessful, mainly due to impermeability of the skin epidermal barrier to hydrophilic proteins. Among these inefficient attempts were some that even used liposomes as protein carriers to expedite protein penetration into the skin dermis (7). Conclusions from those experiments can be summarized as follows: first, protein translocation through the epidermis is a very inefficient process; second, it's not a practical delivery route for therapeutic proteins that need systemic distribution (8). As main objectives of those experiments were to monitor the possibility of systemic protein delivery through the skin, most of the evaluations were made using *in vitro* cell systems based on the amount of proteins that physically translocated the whole span of the skin depth under examination. Thus, in retrospect, these systems could have missed the *in vivo* biological effects caused by proteins in transit or entrapped inside the respective skin. Nontheless some of those early experiments hinted at forthcoming of protein cosmetic ingredients; for example, although penetration of liposomal gamma-interferon through the skin *in vitro* was very inefficient at best (7), it was able to elicit biological response in the form of a secondary effector protein expression *in vivo* (8). Recent studies on the topical delivery of macromolecules recognized the importance of hair follicular or transfollicular route of delivery (9). In this mode of macromolecular delivery, liposomes turn out to be one of the best carriers in which a target molecule can be transported inside the hair follicle (10). It seems dependent on the size, surface charge, flexibility, and, possibly, composition of the liposomes carrying target molecules. In addition, though transport efficiency is probably much less compared to follicular transport, very flexible liposomes, termed transfersomes, claim to be able to pass through stratum corneum via intercellular spaces and/or tight junctions of the skin epidermis, enabling them to reach the viable cells of the epidermal layers (11). As the average diameter of the hGH-encapsulating liposomes can be controlled to around 200nm **(Fig. 6)** and up to 5um has been observed to enter the hair follicles, taken together, it's not difficult to imagine that liposome-encapsulated hGH certainly can enter the skin hair follicles and interact with the outermost viable cells constituting the hair follicles. To detect hGH delivered into hair follicles, we used *N*-terminal poly-histidine-tagged hGH(His-hGH) instead of hGH to avoid cross-reactivity with endogenous mouse

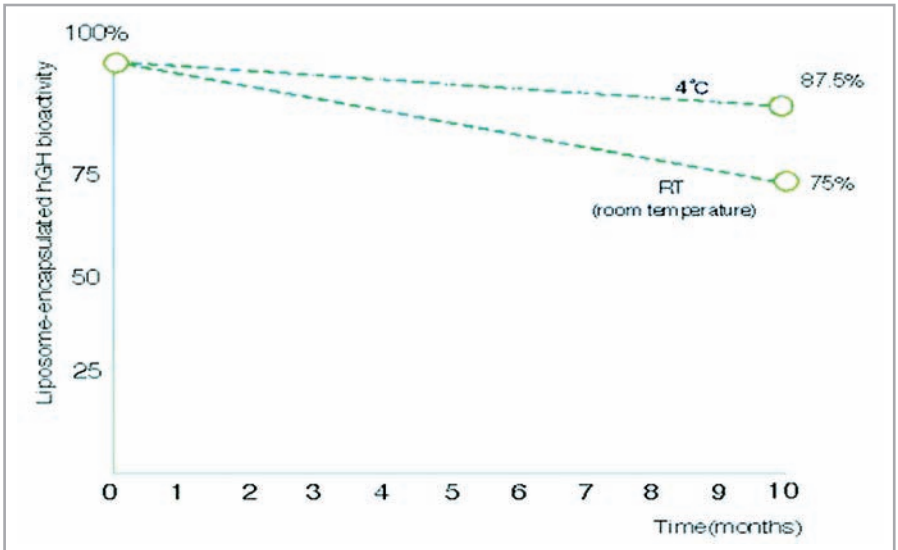


Fig. 5 Stability of a NL-hGH solution

GH. When we used anti-His antibody to detect His-hGH, we could indeed observe that NL-His-hGH but not His-hGH alone was efficiently delivered into hair follicles **(Fig. 7)**. However, it is not likely that macromolecular hGH penetrates the epidermal layer of the hair follicle wall into the dermis, judging from **Fig. 7** as well as considering a plethora of previous reports attesting otherwise.

3.Efficacy issue

For hGH to have any effect on the skin, the skin cells should have receptors for hGH, as hGH supposedly functions through interaction with its receptors on the cell surface. Given that hGH circulates with the blood *in vivo* and therefore can interact only with the cells facing the blood, the finding that hGH receptors are practically all over the cells making up the hair follicles that normally won't directly contact the blood, is quite unexpected (12). Putting available experimental data together, one can come up with a motion picture with the following developments: liposome-encapsulated hGH is applied on the intact skin; hGH-encapsulating liposomes accumulate centering around hair follicles; hGH-encapsulating liposomes enter hair follicles of the skin, slither down along the hair shaft interacting with the environment on the path inside the hair follicles, subsequently release hGH molecules as

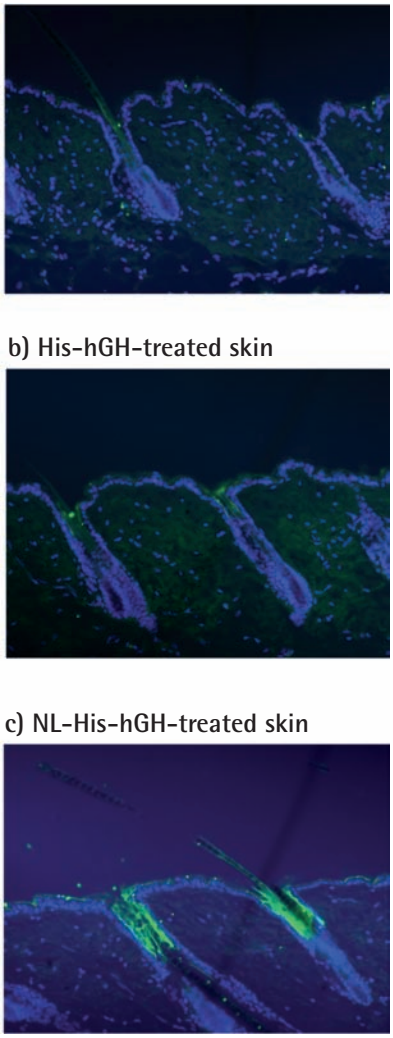


Fig. 7 Follicular delivery of a NL-His-hGH solution

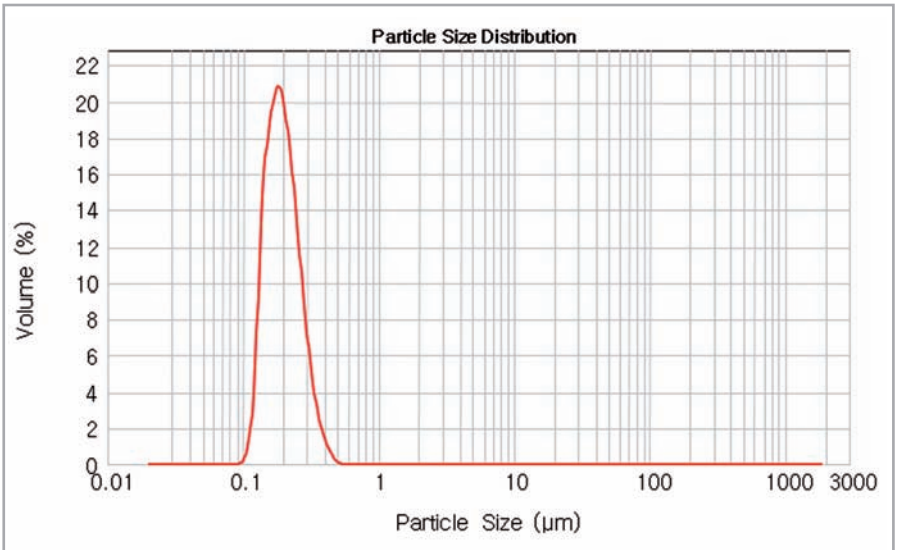


Fig. 6 Size distribution of a NL-hGH solution

the liposomes disintegrate and lose their phospholipid components to the surrounding environment; unshielded hGH molecules interact with hGH receptors expressed on the viable cell surfaces constituting the hair follicular contours; the interacting cells convey signals to themselves and to the adjacent cells that result in observable efficacies, given time; released hGH is exposed to and eventually degraded by abundant proteases present in the hair follicle. Thus, a rational foundation for efficacy testing of liposome-encapsulated hGH is solidly laid.

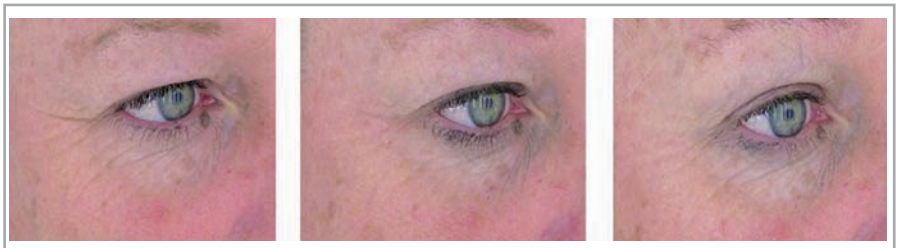
One significant conceptual addition to the aftermath of follicular delivery came from recent studies focused on elucidating the location of the skin stem cells. Traditionally, epidermal basal layer cells were thought to be the stem cells for the epidermal skin. Though the epidermal basal layer seemed to contain its own pool of stem cells, specifically termed »interfollicular stem cells«, giving rise to skin epidermis, it turned out that a small bulge region just under the sebaceous gland in the hair follicle contains the stem cells, dubbed as »bulge stem cells«, that can supply all kinds of skin cells of the epidermis including the hair follicle (13, 14). More specifically, the bulge stem cells are the cells that divides slowly and steadily to give progenitor cells termed »transiently amplifying progenitor cells« that become fast dividing cells possessing a limited proliferative capacity as they migrate toward their presumed destinations and progressively differentiate into epidermal cells, sebaceous gland cells, or hair follicle matrix cells (15). In other words, the hair follicles are where treatments for any fundamental change in the skin cell biology should focus on. Thus, quite by chance, liposome-encapsulated hGH satisfied all the requirements necessary to induce any hGH-prompted change or effect in and on the skin. The followings are some of the *in vivo* experimental results came out of the topical application of a liposome-encapsulated hGH solution to the human or mouse skin.

• *Anti-acne effect*

Anti-acne effect is one of the most pronounced *in vivo* efficacies of nano-lipo-

some-encapsulated hGH(NL-hGH). Anti-acne effect is recognized usually within a week **(Fig. 2)** and has been confirmed by scores of people of differing ages and ethnic backgrounds. One very nice thing about using NL-hGH is that there appears practically no side effect. An effective dose of NL-hGH for an anti-acne effect seems to depend on an individual's race and type of skin under test. Surprisingly, no side effect whatsoever in using excess amount of NL-hGH seems evident. The reason behind the anti-acne effect of hGH is not known, but it might have something to do with the presence of hGH receptors in the sebaceous gland cells resulting in suppression of an auto-inflammatory response of the immune cells of the skin. Recent experiments in our lab with a mouse model of collagen induced arthritis showed for certain that hGH has an anti-inflammatory effect on this mouse model when administered intraperitoneally (*Yoonhee Han, Zungyoon Yang*, and *Dahlkyun Oh*, unpublished results). NL-hGH seems especially effective to a scar-prone acne on the oily skin type and resembles retinoic acids in its anti-acne effect, but without any irritation. As its anti-acne effect is sustained as long as the application is continued, it also seems to exert a prophylactic effect on acne eruptions (*Dahlkyun Oh*, unpublished observations).

• *Anti-wrinkle effect*
Anti-wrinkle effects were tested using a mouse model. Hairless mice were randomly divided into 4 groups of 3 mice each and each group is subjected to the following treatment: 1) no UVB and no treatment; 2) UVB and no treatment; 3) UVB and a NL-hGH treatment; 4) UVB and a liposome-only (NL) treatment.
Mice were exposed in UVB twice a week, 20mJ at a time, for 2 months. These treatments cause distinguishable wrinkles on the backs of the hairless mice by judging with naked eyes. Once wrinkles are formed, NL-hGH treatments were followed for another 2 months with 2 times-a-week protocol. After the 2 months treatments, that is, 4 months after the test was initiated, it was quite obvious that at least two pairs of groups are qiuite easily distinguishable from the other two by naked eyes, that is, a pair consisting of 1) and 3) vs. the pair 2) and 4) above. The results are summarized in **Fig. 4**. Subsequently, an anti-wrinkle effect of NL-hGH on the intact human skin *in vivo* has been circumstantially confirmed, as shown in **Table 1** and **Fig. 8**.



before treatment **4 weeks after treatment** **8 weeks after treatment**

Fig. 8 Reduction of wrinkles by a NL-hGH formulation

• *Skin-whitening/lightening effect*
Significant whitening effects of NL-hGH were observed in human skin tests, including lightening of age spots and UV-induced freckles, as well as overall whitening of dark facial complexion **(Fig. 3** and **Table 1)**. Again the mechanism of whitening effects has yet to be established. One of our hypotheses is that hGH might suppress the conversion of pro-melanocytes to melanocytes occurring at or around the junction of dermis and the epidermis. It is based on the experimental results conducted on a set of artificial skin patches, as hGH seems to fortify some stem cell characters (16) (e.g., heightened expressions of both integrin-α6 and integrin-β1, skin stem cell markers, due to hGH action **(Fig. 9)**) of the epidermal basal layer of the artificial skin around which conversion of premelanocytes to melanocytes seems to occur *in vivo*. In other words, hGH might potentially keep premelanocytes from developing into malanocytes. We also performed experiments to see whether NL-hGH directly exerts an inhibitory effect on the melanin production of *in vitro* cultured mouse B16 melanoma cell-line, using Arbutin as a positive control. The results clearly showed NL-hGH's inhibitory effect on melanin production of mouse B16 melanoma cell-line **(Fig. 10)**.

• *Effect on skin-tone improvement*
Clarity, firmness and moisture contents of the skin can be improved through prolonged use of NL-hGH **(Table 1)**. The level of moisture content seems to be adjusted to normal levels for all skin types, i.e., both dry and oily skin types were benefited from the use of NL-hGH (*Dahlkyun Oh*, unpublished observations). Again, the reasons behind these effects are yet to be elucidated. My inclination is that these might have something to do with hGH's ability to strengthen stem cell characters of the artificial epidermal basal layer cells (see the previous section).

5) Hair-growth effect
All these diverse efficacies of NL-hGH seem to indicate that somehow the skin stem cells of the bulge or transiently amplifying progenitor cells derived from the bulge stem cells in the hair follicle **(Fig. 11)**, are influenced by the presence of exogenous hGH and involved in the pharmaceutical and cosmetic outcomes of the topical NL-hGH application. Based on this rationale, we further examined whether hair (follicle) number or the hair growth cycle of the mouse is affected by the application of NL-hGH, using minoxidil as a positive control. Mice were treated for 2 weeks with NL-hGH after depilation and before peritoneal BrdU injection and subsequently sacrificed 3 hrs after the injection. The results clearly showed that NL-hGH application on the back of the mouse increased not only the hair number but potentially the hair length and thickness. As, in a given hair bulb, a more number of actively prolif-

erating hair matrix cells is correlated with longer duration of anagen phase, thus with longer and thicker hair (17). Minoxidil influenced on the hair number as well, but not the hair length or thickness. These results are summarized in **Fig. 12**. Therefore NL-hGH was superior to minoxidil in increasing both the hair number and possibly the hair length and thickness in the mouse experiments.

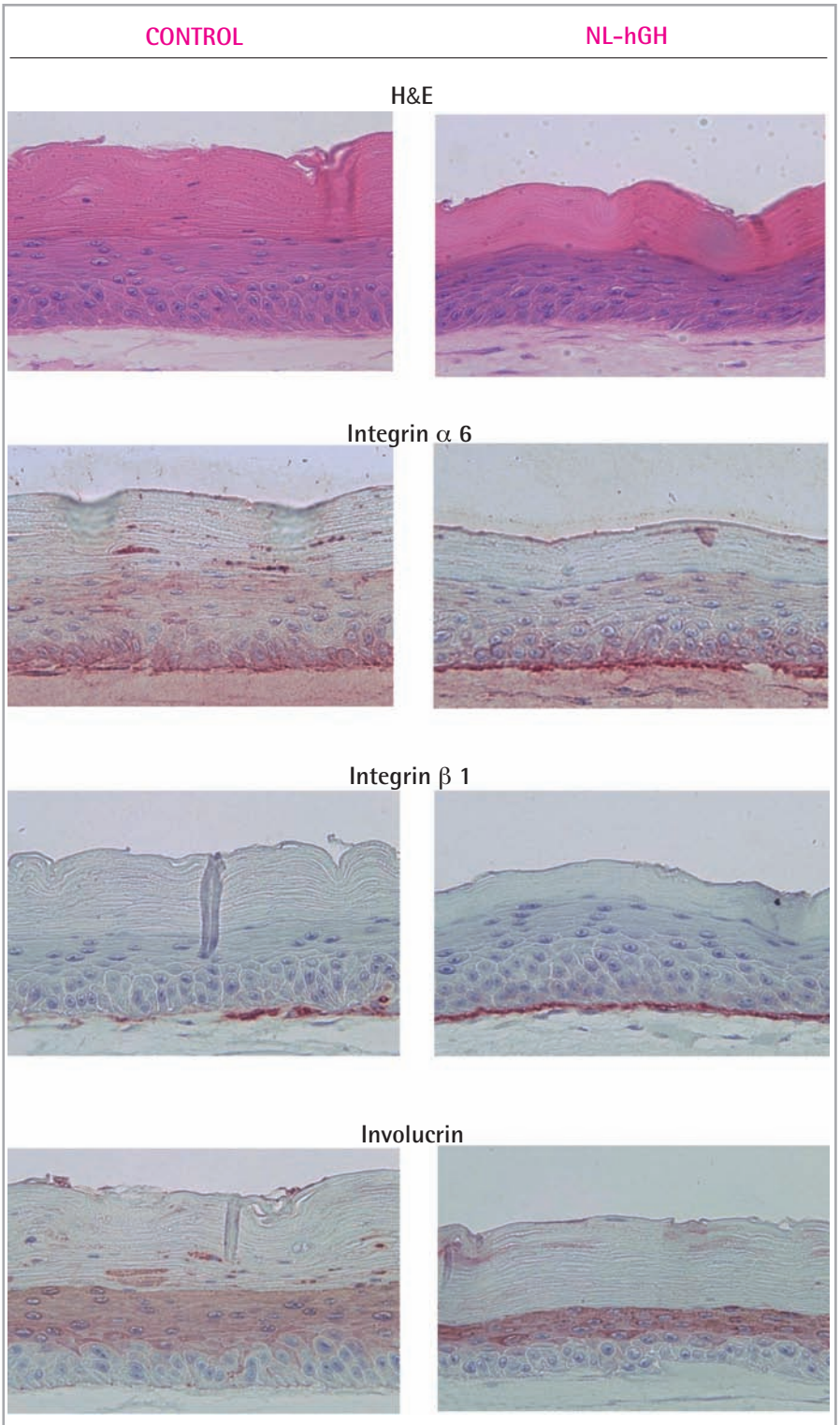


Fig. 9 Effect of a NL-hGH solution on the epidermis and basal layer

One of the frequently rising misgivings in people when they were presented with the workings of the cosmetic NL-hGH has been an issue regarding possibility of NL-hGH to stimulate facial hair growth. No such incident has occurred. In fact, physiological responses of the human scalp hair to sex hormones, for example, are opposite to those of the facial hair in that, whatever stimulates hair growth in the scalp is likely to down-regulate hair growth in the face. Simply look at hairs in men and women on their faces and heads!

4. Safety issue

One of the prevailing preoccupations in the cosmetic field, often implicit, is the notion that »whatever cosmetic ingredient biologically effective to the skin entails some irritation; the more, the severer«. While most, if not all, of the well-known cosmetic active ingredients, including retinoids and AHA's, have fallen within the boundary of this quotation, hGH definitely turned out not to be one of those despite much unfounded concern. In fact, no irritation whatsoever, if properly processed, is the distinctive hallmark of NL-hGH. The rationale behind this assertion can be summarized as follows: 1) hGH is too large a molecule to pass through the epidermal layer of the hair follicle; 2) hGH molecules exposed to inside the hair follicle upon liposome disintegration are subject to rapid protease degradation due to the environment rich in degradative enzymes; 3) the cells having chances of direct interaction with hGH, that is, viable cell layers of epidermis, will most likely undergo apoptosis and naturally be shed from the skin in about 3-4 weeks, thereby leaving no long-term potential aberration to the skin; 4) the concentration of liposome-encapsulated hGH in NL-hGH deliverable to the skin, compared to normal endogenous hGH level in the circulating blood, is such that it won't amount to any physiologically significant systemic entity to the body except the skin under the direct NL-hGH application. Therefore, there is practically no risk of overdosing NL-hGH to the intact skin. About concerns on the nano-toxicity issue, as the nano-sized liposomes in

NL-hGH are made of phospholipids derived from soy or egg lecithin and can be metabolized completely by the cells of hair follicles, unlike those non-degradable metal-containing nano-particles, NL-hGH leaves no potential nano-toxicity. One last thing worth mentioning is the fact that hGH has been one of the most scientifically and medically scrutinized proteins for its potential as a cancer-causing agent, but come out clean of the charges so far (18). According to our experimental results on the artificial skin, quite contrary to the public concerns, it seems to exert a cancer-preventing effect on the skin. That is, although NL-hGH strengthens stem cell character of the epidermal basal layer cells, once the proliferating progeny cells of the basal layer cells are moving away from the basal layer toward the skin surface, NL-hGH seems to help the cells to differentiate to keratinocytes as evidenced by the enhanced expression of involucrin, a differentiation marker **(Fig. 9)**. As cancerous cells can arise from dedifferentiation of terminally differentiated cells, any activity supporting cellular or tissue differentiation can be regarded as an anti-oncogenic activity. Nevertheless, attention should be paid not to apply hGH-containing NL-hGH on the skin of cancer patients, especially on the skin cancer site, as there is not enough evidence that such application can suppress the proliferation of cancerous cells as yet.

5. Formulation and Storage issues

So far, NL-hGH has been formulated into toner, cream, serum, and gel forms and been made to maintain its integrity in terms of the biological activity. Once NL-hGH has been properly formulated, those formulations sometimes seem to turn out more stable than NL-hGH itself (*Dahlkyun Oh*, unpublished results). This is probably due to a »cage effect« in which the water phase containing NL-hGH is divided into tiny droplets and individually firmly surrounded by a hydrophobic water repellent wall, providing safe harbor for NL-hGH particles by limiting their turbulent encounters with one another, hence stabilizing the liposomal shield surrounding hGH. By a similar principle, nanoliposomes surrounded by the wa-

	melanin (µg/ml)	melanin content (%)
Control (NL-hGH 0ng/ml)	44.1	100
Arbutin (100µg/ml)	36.8	83.4
Arbutin (200µg/ml)	30.6	69.3
Liposome-only control corresponding NL-hGH (1µg/ml)	43.4	98.4
NL-hGH (1ng/ml)	35.9	81.4
NL-hGH (10ng/ml)	31.4	71.2
NL-hGH (100ng/ml)	29.4	66.7
NL-hGH (1µg/ml)	22.4	50.8

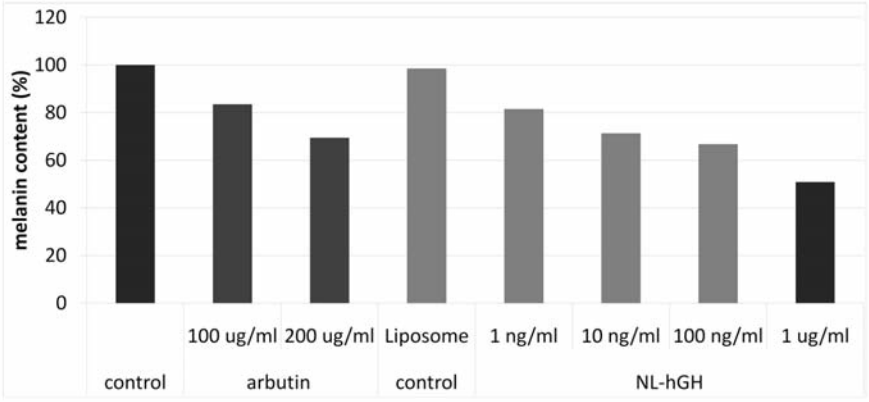


Fig. 10 Effect of a NL-hGH solution on melanin production of mouse B16 melanoma cell line

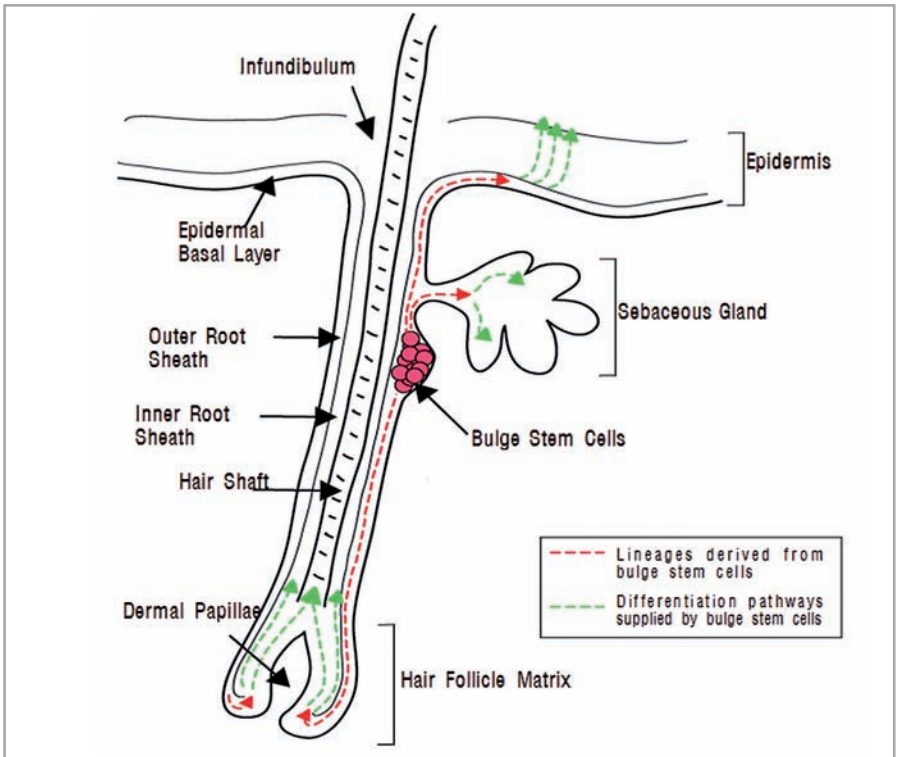
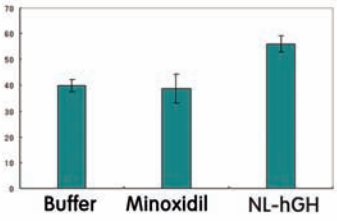


Fig. 11 Schematic diagram delineating differentiation lineages and migration paths of the hair follicle »bulge stem cells« (adopted from 15)

A) Increase of BrdU-stained actively proliferating hair matrix cell counts by NL-hGH treatment



	Average	SE
Buffer	39.9	2.3
3% Minoxidil	38.8	5.7
NL-hGH	56.1	3.1

B) Hair counts per 0.01 mm² using SEM

	DW	Liposome	Minoxidil	hGH	NL-hGH
Average	9.06	9.41	9.82	9.65	11.17
SE	0.48	0.35	0.42	0.29	0.32

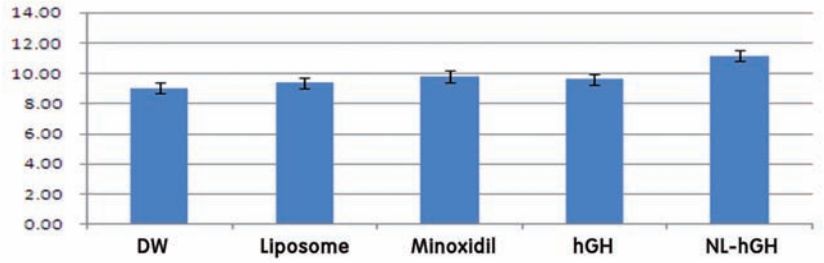


Fig. 12 Effects of a NL-hGH solution on hair growth

ter-soluble polymers can be stabilized due largely to the swelling nature of the polymers in aqueous solution and their accommodating nanoliposomes into sequestration inside polymeric 3-dimensional criss-crossed structures. This extra-stabilizing effect is pronounced and illustrated especially in the fact that some of these formulations kept at the temperature as high as 55 °C can stably maintain hGH bioactivity for a prolonged period of time while NL-hGH itself fails to do so. Thus, it is even more important to take proper precaution not to break a liposomal shield during the formulating processes. Some rules of thumb avoiding a liposome breakage are: 1) keep the mixing temperature above 4 °C but as cool as possible, and if exposure to high temperature is not avoidable, run high temperature mixing operations involving NL-hGH as short and as late as possible; 2) do not put NL-hGH under pH lower than 5 or higher than 10 under any circumstances as it can cause precipitation or dissolution of liposomes, respectively; 3) keep NL-hGH away from direct contact with organic ingredients or solvents that can destabilize liposomes.

■ Conclusion

Traditionally, proteins were not considered to be proper candidates for active cosmetic ingredients mainly due to their intrinsic labile nature in aqueous solution on one hand and our conceptual difficulty in delivering their formidable sizes to the viable skin layers on the other. However, those barriers can be overcome.

To summarize, if a protein were to be a cosmetic active ingredient, it should satisfy the following prerequisites: first, it can be formulated to stay stable at room temperature for at least one year; second, it should be innocuous to the skin and readily available for interaction with the viable cells in the hair follicle; third, the interacting cells in the hair follicle should have receptors for the ligand protein on their surfaces; fourth, the ligand-receptor interaction should lead to long-term beneficial effects on the skin.

The main point of this article is that proteins can, if appropriately chosen and formulated, be the most efficacious yet safest cosmetic ingredients available and that hGH is definitely one of those proteins known to the cosmetic field.

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