

A study of the activity and effectiveness of recombinant fibroblast growth factor (Q40P/S47I/H93G rFGF-1) in anti-aging treatment

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Abstract

Introduction: Fibroblast growth factor 1 (FGF-1) is a powerful mitogen involved in the stimulation of DNA synthesis and the proliferation of a wide variety of cell types. Fibroblast growth factor 1 was genetically modified to improve its thermal stability and resistance to protease degradation without losing its biological activity.

Aim: To study the impact of Q40P/S47I/H93G rFGF-1 on skin cells, its penetration through the skin and the evaluation of the rFGF-1-cosmetic product properties.

Material and methods: *In vitro* studies included the examination of primary fibroblast and keratinocyte viability after the incubation with rFGF-1. The penetration abilities of rFGF-1 in various formulations and carrier systems were examined *ex vivo* by the Raman spectroscopy. *In vivo* studies – HF Ultrasound and 3D Imaging System – were used to evaluate the anti-aging properties of creams containing rFGF-1.

Results: *In vitro* studies demonstrated that rFGF-1 strongly enhanced the viability of the treated cells. The Raman Spectroscopy analysis indicated that rFGF-1 encapsulated in lipid spheres penetrate through the stratum corneum to the depth of 60 µm, and added to the o/w formulation – could penetrate to a depth of 90 µm. The results obtained from Primos revealed the reduction of the volume and the depth of the wrinkles. Changes in the skin structure in the analyzed areas were evaluated by HF Ultrasonography.

Conclusions: Recombinant FGF-1 strongly stimulated fibroblast and keratinocyte proliferation. However, the transition of this protein through the SC required an appropriate carrier system – lipid spheres. All tests – *in vitro*, *ex vivo* and *in vivo* – have proved that rFGF-1 is a substance with a potentially wide spectrum of use.

Key words: HF ultrasonography, Primos, keratinocytes, Raman spectroscopy.

Introduction

Aging results mainly in the loss of dermal collagen and the accumulation of unorganized collagen and elastin fibers in the dermis, which leads to the appearance of the most common signs of aging, i.e. wrinkles, elastosis and loss of skin tone. Fibroblasts play the key role in wrinkle formation because they produce basic structural skin substances: collagen, elastin and hyaluronic acid. These substances provide the skin with appropriate elasticity, tensile strength, firmness (collagen and elastin fibers) and moisturization (hyaluronic acid). During the aging process, the proliferative and metabolic activity of fibroblasts decreases, the fibers' functions are impaired and

their structure becomes modified and then destroyed. Some of the biochemical effects of intrinsic and extrinsic skin aging are similar to the formation of a wound [1]. Human growth factors (HGF) play the key role in wound healing and they are important for proper cell functionality [2]. Human growth factors participate in cell division, new cell and blood vessel growth as well as in collagen and elastin production and distribution. They are also involved in the growth and regeneration of many different cells in the body, including fibroblast, liver, vascular, thyroid, ovary and pituitary gland cells. Nowadays, a lot of research is conducted in order to evaluate the usefulness of HGFs in anti-aging treatment.

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Human acidic fibroblast growth factor 1 (FGF-1) belongs to a large family of growth factors that bind to transmembrane receptors with a cytoplasmic tyrosine kinase domain. The FGF-1 is a powerful mitogen involved in the stimulation of DNA synthesis and proliferation of a wide variety of cell types. It plays important roles in various stages of development and morphogenesis, as well as in angiogenesis and wound healing processes [3, 4].

Fibroblast growth factor 1 was genetically modified to improve its thermal stability and resistance to protease degradation without losing its biological activity. The triple mutant Q40P/S47I/H93G has changes in the sequence localized in the loop between residues Leu89 and Asn95 (mutation H93G), within residues Thr34-Ile42 (Q40P) and in strand 4 (S47I) (Figure 1). The obtained protein was fully functional and characterized by higher mitogenic activity, a longer half-life and much greater resistance to proteolysis than the wild type [5, 6].

Aim

The aim of this research was to investigate the impact of rFGF-1 on skin cells, its penetration abilities through the stratum corneum and to evaluate the anti-aging properties of cosmetic products containing Q40P/S47I/H93G rFGF-1 as an active compound.

Material and methods

In vitro study

In vitro studies were carried out on two types of skin cells: fibroblasts (human primary fibroblasts from donors of different age: 20, 34, 40 and 60 years of age) and keratinocytes: KB (ATCC) and HaCaT lines (Deutsches Krebsforschungszentrum Stabsstelle Technologietransfer Heidelberg, Germany) [7].

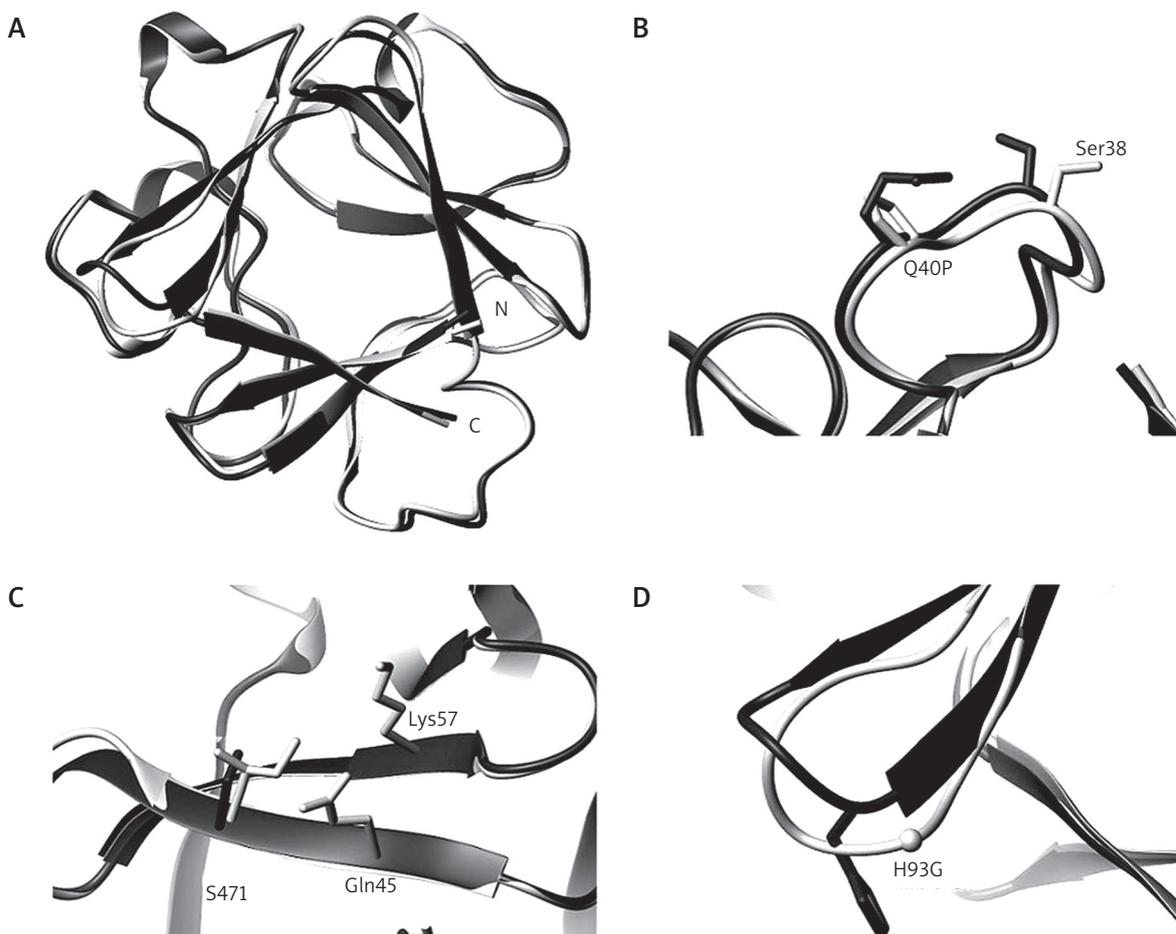


Figure 1. A – Superposition of wild-type FGF-1 (PDB code 1rg8) and the Q40P/S47I/H93G mutant (PDB code 2q9x). Regions of mutated residues are shown in (B) (Q40P), (C) (S47I) and (D) (H93G). The wild-type structure is shown in dark grey and that of the triple mutant in light grey [6]. Reproduced with permission of the International Union of Crystallography

Fibroblasts and the HaCaT cell lines were grown in standard MEM medium (Eagle's), (GIBCO) and the KB cells were cultured in RPMI medium. Cells were stimulated by recombinant fibroblast growth factor 1 (rFGF-1) [5, 6] in 2 concentrations of 10 ng/ml and 100 ng/ml. Cell viability was determined by an MTT assay after 24 h of stimulation (KB and HaCaT lines) and after 7 days of stimulation (fibroblasts).

Ex vivo study – Raman spectroscopy

Raman measurements were carried out on dried samples using a Renishaw inVia Raman system equipped with a 100-mW laser emitting a 632.8 nm line used as the excitation source. The light from the laser was passed through a line filter and focused on a sample mounted on an X-Y-Z translation stage with a 50× microscope objective. The Raman-scattered light was collected by the same objective through a holographic notch filter to block out Rayleigh scattering. A 1800 groove/mm grating was used to provide a spectral resolution of 5 cm⁻¹. The Raman scattering signal was recorded by a 1024 × 256 pixel RenCam CCD detector. The beam diameter was approximately 2.5 μm. Typically, the spectra were acquired for 10–30 s, either in a static mode, centering at 1200 cm⁻¹, or in an extended mode, between 300 and 3400 cm⁻¹, with the laser power measured at the sample being 5 mW. The spectra were normalized by the laser power and the collection times. This stage of the research was carried out in three phases. Firstly, the determination of the fingerprint (reference spectra) for the recombinant FGF-1 was done. Then, the evaluation of the penetration ability of pure rFGF-1, pure rFGF-1 encapsulated in liposomes and lipid spheres [8], pure rFGF-1 added to standard o/w and w/o emulsions, and finally the composition of rFGF-1 and lipid spheres from o/w and w/o emulsions was carried out. Fingerprints of pure substances were generated at a depth of 15–25 μm. All samples were applied on skin explants (10 μl each) from female donors (23–26 years of age) and incubated for 72 h on metal strainers. Those skin explants were used only to investigate the skin system penetration.

In vivo study

The study sample comprised 25 women in the age range between 51 and 59 with visible signs of aging. All the volunteers signed the consent to participating in the test. The enrolled women applied the anti-wrinkle creams day and night for 4 weeks. Both creams included the active ingredient – rFGF-1 in lipid spheres.

The skin evaluation was performed twice – prior to and after the 4-week treatment. The High Frequency Ultrasound (HFU) device EPISCAN Ultrasound Scanner Version 4.0.0.030 (Longport International Ltd., USA), equipped with the 50 MHz mechanical probe and Primos 3D Imaging System (GFMesstechnik GmbH, Germany)

was used for the examinations. All scans and images were performed at the same location: the nasolabial fold and the wrinkle between the eyebrows, at fixed settings of the scanner's and camera's parameters. The following parameters were investigated: epidermis echo thickness, dermis thickness, dermis echogenicity. Separately, the echogenicity of the dermis inferior (reticular) layer (HFU) [9] and the depth and volume of measured wrinkles (Primos) [10] were tested. The study was approved by the ethical committee.

Statistical analysis

The sample distribution was determined by the W Shapiro-Wilk test and the statistical significance for dependent samples by the *t*-Student test, while for the non-parametric by the Wilcoxon test. $\alpha < 0.05$ was considered statistically significant (Statistica software ver. 10).

Results

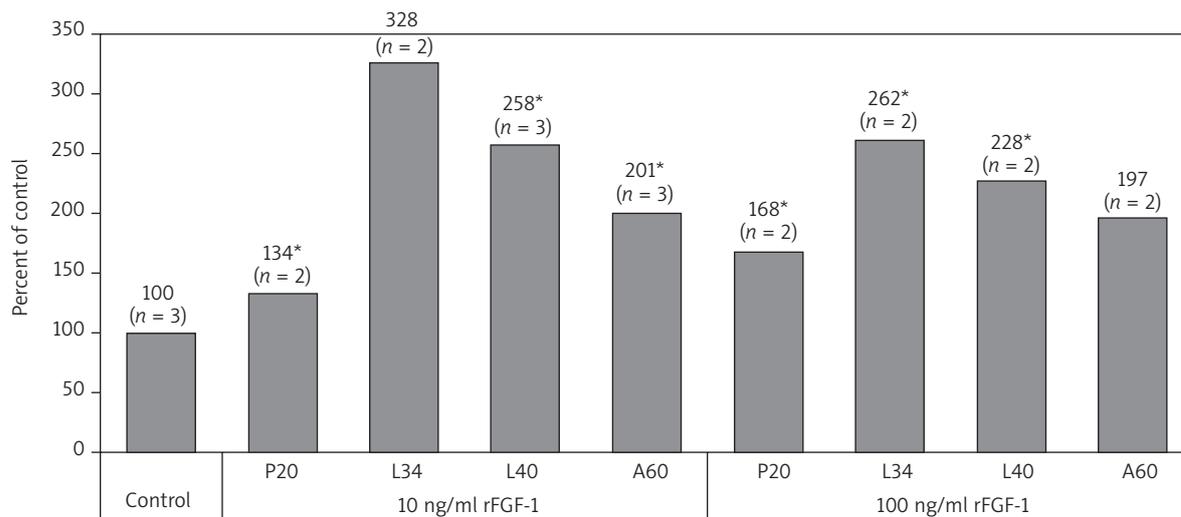
In vitro study

The studies carried out on fibroblasts revealed that Q40P/S471/H93G rFGF-1 strongly enhanced the viability of cells from each donor. The strength of stimulation depended on the cell line. rFGF-1 most effectively stimulated the proliferation of fibroblasts from donors of 34 and 40 years of age (Figure 2). In almost all samples better results were achieved after stimulation with 10 ng/ml rFGF-1. However, in P20 cells higher proliferation was observed after stimulation with 100 ng/ml rFGF-1.

rFGF-1 stimulated the proliferation of keratinocytes from the KB line, as from the HaCaT line (Figure 3). In the KB line there was a statistically significant increase of 25% (10 ng/ml rFGF-1) and of 13% (100 ng/ml rFGF-1) in cell viability. In the HaCaT line there was an improvement in cell viability of about 42% after stimulation with 10 ng/ml rFGF-1, and 11% after stimulation with 100 ng/ml rFGF-1 (Figure 3).

Ex vivo study – Raman spectroscopy

The fingerprint of rFGF-1 was generated. Q40P/S471/H93G rFGF-1 does not penetrate through the stratum corneum as a pure substance, or from the w/o and o/w formulations (Figure 4). This was evidenced by the lack of bands at frequencies: 1063, 1131, 1291, 1440, 2850 and 2883 cm⁻¹ characteristic for rFGF-1. rFGF-1 enclosed in liposomes did not penetrate through the stratum corneum either (Figure 5). However, rFGF-1 encapsulated in lipid spheres (SLNs) was able to migrate through the skin to a depth of 20 μm (Figure 6). Moreover, the rFGF-1 in lipid spheres can migrate through the dermis to a depth of 60 μm (Figure 7) and it was not present in the deeper layers but it can penetrate to a depth of 90 μm from the o/w formulation containing 5% of the composition of



Cell line	Concentration of rFGF-1	Mean value (OD, λ = 595 nm)	Standard deviation	Difference	Standard deviation of difference	P-value
P20	Control	0.2435	0.01343	-0.0835	0.00919	0.04946
	10 ng/ml	0.3270	0.02263			
	Control	0.2435	0.01343	-0.1660	0.01697	0.04594
	100 ng/ml	0.4095	0.03040			
L34	Control	0.1975	0.00495	-0.4510	0.07212	0.07169
	10 ng/ml	0.6485	0.07707			
	Control	0.1975	0.00495	-0.3197	0.00707	0.00995
	100 ng/ml	0.5175	0.01202			
L40	Control	0.2223	0.01817	-0.3514	0.07674	0.01553
	10 ng/ml	0.5737	0.07998			
	Control	0.2195	0.02475	-0.2805	0.02616	0.04193
	100 ng/ml	0.5000	0.05091			
A60	Control	0.3403	0.07656	-0.3422	0.05840	0.00956
	10 ng/ml	0.6827	0.09903			
	Control	0.3050	0.06505	-0.2945	0.06435	0.09759
	100 ng/ml	0.5995	0.00071			

Figure 2. The mean viability of human primary fibroblasts from 20- (P20), 34- (L34), 40- (L40) and 60- (A60) year-old donors (MTT assay) after 7-days' stimulation by rFGF-1 in concentrations of 10 or 100 ng/ml. Untreated cells were used as a control. *Statistically significant results ($p < 0.05$), (Statistica software ver. 10); table contains the RAW data (OD)

rFGF-1 and lipid spheres (Figure 7). An analogous test was done for the formulation w/o, but there were no bands characteristic for the studied active ingredient.

In vivo study

The visual analysis of the collected ultrasound images was done by three independent researchers separately for each individual parameter. The analysis of results has produced a statistically significant increase in dermis thickness in the wrinkle between the eyebrows (forehead area). Before applying the cream the average

thickness of the dermis (measured on the forehead) was 2.03 mm. After 4 weeks it increased to 2.23 mm, indicating a 10.01% improvement in this parameter. However, there were no similar results observed at the nasolabial fold (Table 1). For the epidermis echo thickness differences between pre- and post-treatment values were observed in both measured areas (Table 1), but they were not statistically significant. There was also a change involving the increase in the echogenicity of the dermis inferior layer. The pre- and post-treatment values of this parameter showed statistically significant differences

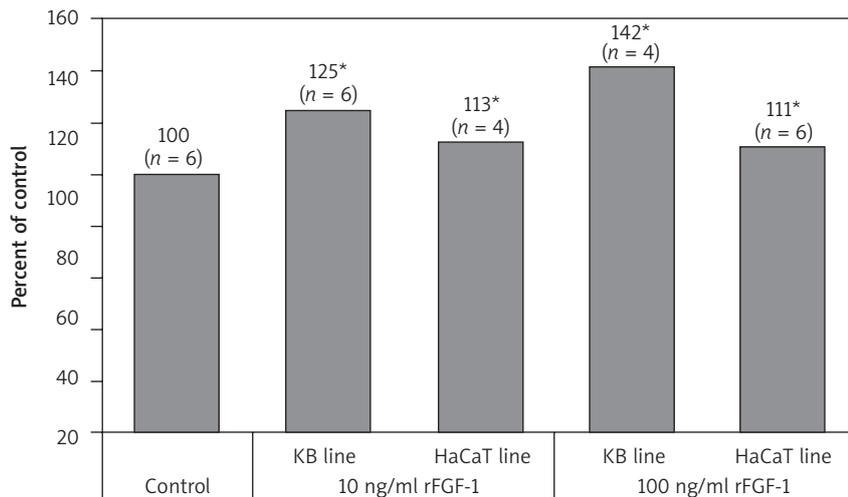


Figure 3. The mean viability of keratinocytes: KB and HaCaT lines (MTT assay) after 24 h' stimulation by rFGF-1 in concentrations of 10 or 100 ng/ml. Untreated cells were used as a control. *Statistically significant results ($p < 0.05$) (Statistica software ver. 10); table contains the RAW data (OD)

Cell line	Concentration of rFGF-1	Mean value (OD, $\lambda = 595 \text{ nm}$)	Standard deviation	Difference	Standard deviation of difference	P-value
KB	Control	0.5102	0.03789	-0.1255	0.05470	0.00242
	10 ng/ml	0.6357	0.03049			
KB	Control	0.4882	0.02133	-0.2043	0.01352	0.00008
	100 ng/ml	0.6925	0.01836			
HaCaT	Control	0.1562	0.00486	-0.0203	0.00411	0.00223
	10 ng/ml	0.1765	0.00311			
HaCaT	Control	0.1587	0.00532	-0.0180	0.00498	0.00031
	100 ng/ml	0.1767	0.00356			

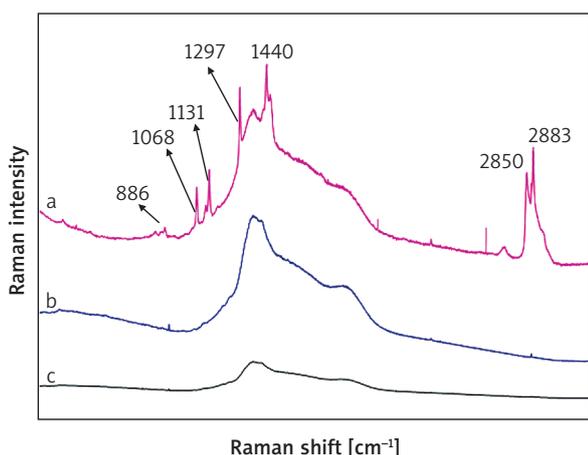


Figure 4. The spectra of: a) pure rFGF-1 (10 µg/ml) – fingerprint, b) w/o emulsion with pure rFGF-1 (100 ng/ml), c) o/w emulsion with pure rFGF-1 (100 ng/ml). Lack of bands at frequencies of 1063, 1131, 1291, 1440, 2850 and 2883 cm^{-1} – characteristic for the rFGF-1

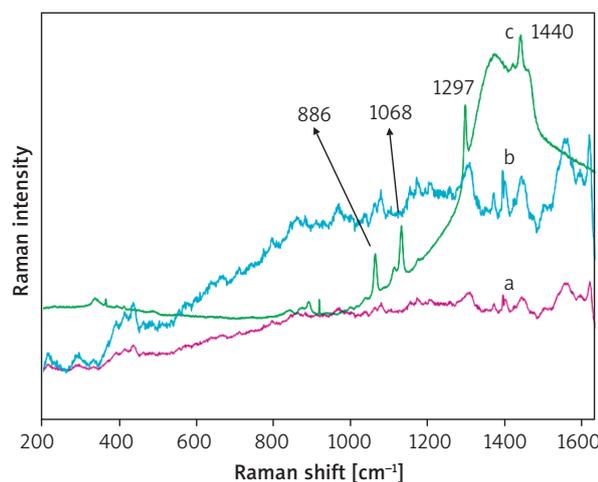


Figure 5. Migration of the rFGF-1 enclosed in liposomes. The spectra of: a) liposomes, b) rFGF-1 enclosed in liposomes (0.8 µg/ml), c) pure rFGF-1 (10 µg/ml). For spectrum b there are mainly bands characteristic for the liposome and not for the rFGF-1. No bands at frequencies: 1063, 1131, 1291, 1440, 2850 and 2883 cm^{-1} which are characteristic for that active ingredient show that it cannot migrate through the stratum corneum

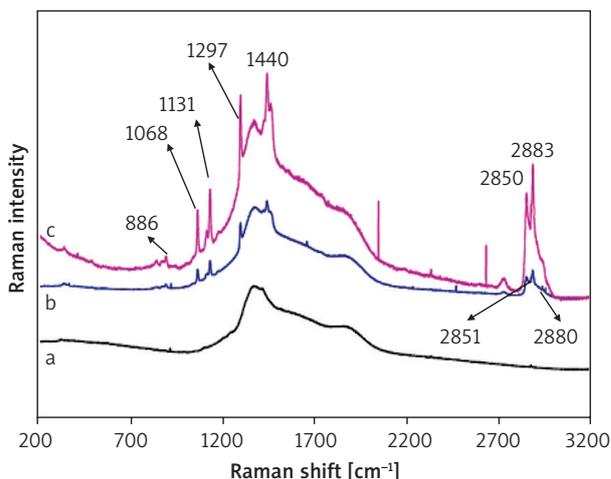


Figure 6. Migration of rFGF-1 encapsulated in lipid spheres (SLNs). The spectra of: a) lipid spheres (SLNs), b) rFGF-1 encapsulated in lipid spheres (SLNs) (0.8 µg/ml), c) pure rFGF-1. The presence of the bands in spectrum b characteristic for the test component (at frequencies: 1063, 1131, 1291, 1440, 2850 and 2883 cm⁻¹) is a clear indication of its presence in the skin sample. rFGF-1 enclosed in lipid spheres passes through the stratum corneum into the living layers

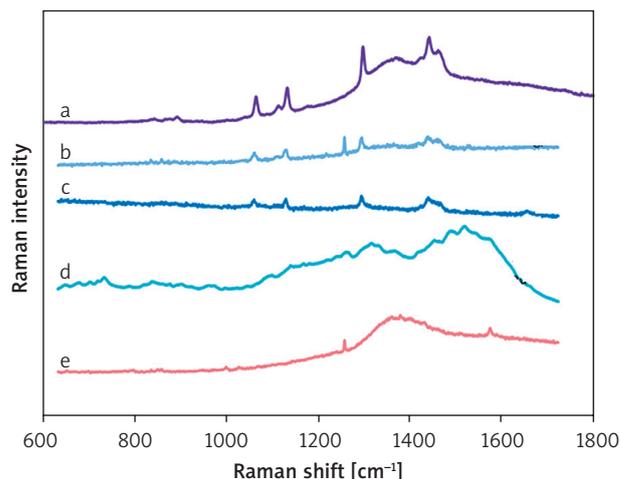


Figure 7. The spectra of: a) pure rFGF-1, b) pure rFGF-1 in lipid spheres (10 µg/ml) – presence of the bands characteristic for the rFGF-1: 1063, 1131, 1291, 1440, 2850 and 2883 cm⁻¹ at a depth of 60 µm, c) 5% rFGF-1 in lipid spheres in the o/w formulation – presence of the bands characteristic for the rFGF-1 at a depth of 90 µm, d) empty lipid spheres – placebo, e) phosphate buffered saline (PBS)

Table 1. Results obtained from HFU

Location	Parameter	Before treatment (mean value)	After treatment (mean value)	Percent of improvement
Nasolabial fold (n = 20)	Epidermis echo thickness [mm]	0.1050	0.1185	+12.86%
	Dermis thickness [mm]	1.8876	1.8857	No improvement
	Dermis echogenicity [pixels]	40705	40023	No improvement
	Echogenicity of dermis inferior layer [pixels]	15930	16130	+1.25%*
The wrinkle between the eyebrows (n = 20)	Epidermis echo thickness [mm]	0.1273	0.1387	+8.96%
	Dermis thickness [mm]	2.0302	2.2335	+10.01%*
	Dermis echogenicity [pixels]	40368	40009	No improvement
	Echogenicity of dermis inferior layer [pixels]	15741	15801	+0.38%*

n – number of women in the test; *statistically significant values.

Table 2. Results obtained from Primos

Location	Parameter	Before treatment (mean value)	After treatment (mean value)	The difference between pre- and post-treatment values
Nasolabial fold (n = 20)	The depth of the wrinkle [µm]	Not available	Not available	-75.70
	The volume of the wrinkle [mm ³]	21.20	18.82	-2.38
The wrinkle between the eyebrows (n = 20)	The depth of the wrinkle [µm]	Not available	Not available	-50.99
	The volume of the wrinkle [mm ³]	6.44	5.48	-0.96*

n – number of women in the test, *statistically significant values, "not available" means that the software gives the result as the difference, without before and after values.

of about 1.25% for the nasolabial location (Table 1). The measurement of dermis echogenicity indicated no statistically significant difference before the application of the products and after the 4-week treatment (Table 1).

The results obtained from Primos revealed differences for both the depth and the volume of the measured wrinkles. At the nasolabial fold a decrease by 75.70 μm (Table 2) in the depth was observed after the use of the set of products. Similar results were observed in the forehead area, where the depth of the wrinkle between the eyebrows decreased by 50.99 μm (Table 2). The volume of the nasolabial fold was 21.20 mm^3 before the test, and it decreased to 18.82 mm^3 at the end of the treatment (Table 2). A similar tendency was observed for the mean value of the volume of the wrinkle between the eyebrows, which was 6.44 mm^3 , whereas after the treatment it decreased to 5.48 mm^3 (Table 2). The difference of about 0.96 mm^3 was statistically significant for the forehead area, but not for the nasolabial fold, where the difference in the volume was 2.38 mm^3 (Table 2).

Discussion

The use of growth factors is a novel anti-aging strategy to rejuvenate and reverse the signs of skin photo-aging. The knowledge and understanding of the role of growth factors in wound healing may predict their role in remodeling the skin's infrastructure and rejuvenating aging skin [2].

Efficacy of rFGF-1 on human skin cells

The rFGF-1, due to its mitogenic functions, has become a potential active ingredient with a wide range of uses in cosmetology directed at skin regeneration. There are some studies reporting that growth factors are a potent antioxidants that inhibits the aging of skin cells, strengthens its defense processes and reduces wrinkles [1]. Some study reports that growth factors are critical for the proliferation of skin cells like keratinocytes and fibroblasts. Zhang *et al.* tested fibroblast growth factor-peptide (FGF-P) as a potential mitigator of radiation effects through the proliferation and the barrier function of keratinocytes. Proliferation is critical in maintaining a healthy barrier layer of keratinocytes. As it was proved, radiation reduced MTT activity in a dose-dependent manner, which was reversed by treatment with FGF-P [11]. Similar results were found by Anitua *et al.* who tested plasma rich in growth factors (PRGF-Endoret) on primary human cells, including keratocytes and conjunctival fibroblasts. In both cell types, the PRGF-Endoret enhanced proliferation [12]. The *in vitro* studies presented in this publication have demonstrated similar effects on human keratinocytes and fibroblasts. The results showed a strong impact of rFGF-1 on the viability of keratinocytes both from KB and HaCaT lines (Figure 3) and fibroblasts from donors

of different age. rFGF-1 most strongly stimulated the proliferation of L34 and L40 fibroblasts (Figure 2). These results suggest that it has strong regenerative properties which can be used in cosmetic products.

Penetration through the skin barrier

Small molecules, due to their physico-chemical properties, have a greater ability to penetrate the skin. In the case of large particles, the barrier of percutaneous absorption is primarily the stratum corneum. Penetration of cosmetic ingredients can be accelerated by either removing the hydrophilic film from the skin surface by surfactants, or by using penetration enhancers like alcohols and glycols [13].

The skin is the critical surface barrier of the body. The structure and lipophilic properties of the outer layer of the skin (stratum corneum) cause only lipophilic, non-polar (non-ionic) and low molecular weight (< 500 Da) molecules to migrate to the deeper layers of the skin, although penetration of such compounds is often insufficient to produce the desired effect [14, 15]. Therefore, there is a need to increase or even to allow the migration of both lipophilic and hydrophilic active substances into the skin [15]. For that reason, nowadays the use of nano- and micro-lipid spheres in cosmetology is growing. The lipid-based formulations seem to be the most appropriate ones for the topical application of active substances because they contain physiological and biodegradable lipids which have affinity with the stratum corneum. When applied externally they do not affect its function [16].

The rFGF-1 is a large protein of 15.196 kDa. Its isoelectric point is 8.12, and it has a positively charged patch on the surface [17]. Due to the large size of this protein there was a necessity to check its penetration abilities through the stratum corneum. To assess the depth of Q40P/S47I/H93G rFGF-1 penetration, the Raman Spectroscopy was used. This method allows the testing of water, NMF, urea, lipids, and carotenoids content in the skin. It is also used for medical applications like analysing the composition of kidney stones, dispensing of medicines, the study of DNA damage [18] and analysis of cancer tissues [19].

Raman analysis provided evidence that both pure rFGF-1, pure rFGF-1 in w/o and o/w formulations and rFGF-1 enclosed in liposomes do not penetrate through the stratum corneum (Figures 4 and 5). Only rFGF-1 enclosed in lipid spheres passes through the stratum corneum, as evidenced by the presence of bands characteristic for the tested component in the generated spectra (Figure 6). After receiving these results the lipid spheres were selected as the dermal transport system for rFGF-1. As shown in these data the ingredients of o/w formulation could act as enhancers and caused rFGF-1 to penetrate deeper (to a depth of 90 μm into the skin) than the composition of rFGF-1 and lipid spheres, which migrated to a depth of 60 μm (Figure 7), what definitely needs further investigation. A feature of lipid particles (lipid spheres)

particularly important from a cosmetology point of view is the creation of a discontinuous occlusive layer on the skin's surface. If the particle size is smaller, their adhesion to the skin is stronger and the occlusion is more effective. This reduces transepidermal water loss, thereby increasing the hydration and elasticity of the skin. Occlusion also has a positive impact on the penetration of active substances into the skin. This has been proven for the Coenzyme Q10, which was applied in the form of a suspension of lipid nanoparticles [20].

The rFGF-1 in lipid spheres passes through the stratum corneum presumably on the basis of forming by lipid spheres an occlusion layer specific for the active substance, which strongly increases its penetrative abilities, and electrostatic interactions between the protein molecules and lipid spheres, where lipid spheres act as a carrier for cosmetic active ingredients which has an affinity with the stratum corneum lipids. On the other hand, it has been suggested that growth factors and cytokines can penetrate through hair follicles, sweat glands or compromised skin, followed by interaction with cells in the epidermis, such as keratinocytes, to produce signaling cytokines that affect cells deeper in the dermis, such as fibroblasts [1].

In vivo efficacy

Several cosmetic products containing human growth factors are currently available on the market. For a few of them clinical results available indicate that the topical application of human growth factors provides beneficial effects in reducing the signs of facial skin aging [2, 21–23]. In the study conducted by Fitzpatrick and Rostan, the mixture of growth factors, cytokines and soluble matrix proteins secreted by cultured neonatal human fibroblasts reduced fine lines, wrinkles and periorbital photodamage. Measurements of Grenz-zone collagen and epidermal thickness measured from the biopsy showed a 37% increase in Grenz-zone collagen and a 30% increase in epidermal thickness [21]. Similar results were obtained by Gold *et al.* who tested a proprietary growth factor and cytokine mixture extracted from cultured first trimester fetal human dermal fibroblasts in moisturizing cream on 18 patients for 60 days. The findings revealed an improvement in the average wrinkle score in periorbital (17%) and perioral (13%) areas [23]. The third product contained liposome-encapsulated transforming growth factor β 1 (TGF- β 1), L-ascorbic acid and black cohosh (*Cimicifuga racemosa*) extract in a silicone base. The study proved an improvement in the wrinkle score for TGF- β 1-containing cream as compared to cream with only L-ascorbic acid and black cohosh extract [22]. All the cited studies are similar to the results of our *in vivo* tests. The instrumental assay provided evidence that the 4-week use of 2 products with the composition of rFGF-1 and lipid spheres (for day and for night) decreases both the volume and the depth of the measured wrinkles (Table 2). Moreover, the exami-

nation made by high frequency ultrasound (HFU) demonstrated changes in the structure of the skin, which may be caused by collagen restoration and better hydration of the tested areas. However, there were dissimilarities depending on the location of measurements. The better results obtained at the nasolabial fold may be caused by the differences in the thickness of the skin, which is thicker on the forehead and thus hinders the penetration of active ingredients.

All the collected data significantly suggest that Q40P/S47I/H93G rFGF-1 has great potential for use in cosmetic anti-wrinkle treatment. Its strong effect on cell proliferation implies that rFGF-1 may have much wider applications, which inevitably must be further investigated.

Conclusions

Q40P/S47I/H93G rFGF-1 strongly stimulated fibroblast and keratinocyte proliferation, which suggests the high potential for the use of this protein in anti-aging skin care products. However, the transition of this protein through the stratum corneum required a suitable carrier system – lipid spheres. Only the use of this form of active ingredient could provide the desired effect.

Growth factors play an important role in reversing the effects of skin aging mediated both by chronological and environmental factors [1]. Active substances such as exogenous growth factors may be used as a strategy to attenuate the signs of aging. Growth factors may manipulate the fundamental mechanisms, e.g. reverse the activity of fibroblasts and stimulate them to increase proliferation [24], which has been proven in the conducted studies. All tests – *in vitro*, *ex vivo* and *in vivo* – confirmed that Q40P/S47I/H93G rFGF-1 is a substance with a potentially wide spectrum of use in cosmetology and wound healing.

Conflict of interest

The authors declare no conflict of interest.

References

1. Mehta RC, Fitzpatrick RE. Endogenous growth factors as cosmeceuticals. *Dermatol Ther* 2007; 20: 350-9.
2. Hilling C. Human growth factors as natural healers: current literature and application. *Cosmetics and Toiletries* 2010; 125: 73-7.
3. Zakrzewska M, Krowarsch D, Wiedlocha A, et al. Design of fully active FGF-1 variants with increased stability. *Protein Eng Des Sel* 2004; 17: 603-11.
4. Nedoszytko B, Sokołowska-Wojdyło M, Ruckemann-Dziurdzińska K, et al. Chemokines and cytokines network in the pathogenesis of the inflammatory skin diseases: atopic dermatitis, psoriasis and skin mastocytosis. *Adv Dermatol Allergol* 2014; 31: 84-91.
5. Zakrzewska M, Krowarsch D, Wiedlocha A, et al. Highly stable mutants of human fibroblast growth factor-1 exhibit enhanced biological activity. *J Mol Biol* 2005; 352: 860-75.

6. Szlachcic A, Zakrzewska M, Krowarsch D, et al. Structure of highly stable mutant of human fibroblast growth factor 1. *Acta Cryst* 2009; D65: 67-73.
7. Boukamp P, Petrussevska RT, Breitkreutz D, et al. Normal keratinization in a spontaneously immortalized aneuploid human keratinocyte cell line. *J Cell Biol* 1988; 106: 761-71.
8. WIPO ST 10/C PL400804.
9. Mlosek R, Malinowska S, Dębowska R, et al. The use of high frequency ultrasound imaging in skin moisturization measurement. *Skin Res Technol* 2013; 19: 169-75.
10. Dzwigałowska A, Sołyga-Żurek A, Dębowska R, Eris I. Preliminary study in the evaluation of anti-aging cosmetic treatment using two complementary methods for assessing skin surface. *Skin Res Technol* 2013, 19: 155-61.
11. Zhang K, Tian Y, Yin L, et al. FGF-P improves barrier function and proliferation in human keratinocytes after radiation. *Int J Radiat Oncol Biol Phys* 2011; 81: 248-54.
12. Anitua E, Sanchez M, Merayo-Lloves J, et al. Plasma rich in growth factors (PRGF-Endoret) stimulates proliferation and migration of primary keratocytes and conjunctival fibroblasts and inhibits and reverts TGF-beta1-induced myodifferentiation. *Investig Ophthalmol Visual Sci* 2011; 52: 6066-73.
13. Morrow DIJ, McCarron PA, Woolfson AD, Donnelly RF. Innovative strategies for enhancing topical and transdermal drug delivery. *Open Drug Deliv J* 2007; 1: 36-59.
14. Orłowski P, Krzyżowska M, Winnicka A, et al. Toxicity of silver nanoparticles in monocytes and keratinocytes: potential to induce inflammatory reactions. *Centr Eur J Immunol* 2012; 37: 123-30.
15. Cal K. Across the skin barrier: known methods, new performances. In: Caldwell GW, ur-Rahman A, Yan Z, Choundhary MI. *Frontiers in drug design and discovery*. Bentham Science Publishers 2009; 4: 162-88.
16. Souto EB, Muller RH. Cosmetic features and applications of lipid nanoparticles (SLN, NLC). *Int J Cosmet Sci* 2008; 30: 157-65.
17. Zakrzewska M. Mutational analysis of human acidic fibroblast growth factor –FGF1. PhD Work, University of Wrocław, Faculty of Natural Science 2005.
18. Caspers PJ, Lucassen GW, Puppels GJ. Combined in vivo confocal Raman spectroscopy and confocal microscopy of human skin. *Biophys J* 2003; 85: 572-80.
19. Pacholczyk B, Fabiańska A, Kusińska R. Analysis of cancer tissues by means of spectroscopic methods. *Contemp Oncol* 2012; 16: 290-4.
20. Muller RH, Petersen RD, Hommoss A, Pardeike J. Nanostructured lipid carriers (NLC) in cosmetic dermal products. *Adv Drug Deliv Rev* 2007; 59: 522-30.
21. Fitzpatrick RE, Rostan EF. Reversal of photodamage with topical growth factors: a pilot study. *J Cosmet Laser Ther* 2003; 5: 25-34.
22. Ehrlich M, Rao J, Pabby A, Goldman MP. Improvement in the appearance of wrinkles with topical transforming growth factor beta(1) and 1-ascorbic acid. *Dermatol Surg* 2006; 32: 618-25.
23. Gold MH, Goldmann MP, Biron J. Efficacy of novel skin cream containing mixture of human growth factors and cytokines for skin rejuvenation. *J Drug Dermatol* 2007; 6: 197-201.
24. Rhein RD. Aging skin – general considerations. In: *Aging skin: current and therapeutic strategies*. Rhein LD, Fluhr J (eds). Allured Business Media 2010.